Remarks

Claims 1, 5, and 7-8 are currently pending in this application. No claim amendments are made in this paper, and thus, no new matter has been added.

Applicants respectfully submit that all of the pending claims are allowable for at least the following reasons.

A. Claims 1 and 7 Are Patentable

At the outset, Applicants respectfully point out that, regardless of what is set forth in the Office Action, claims 1 and 7 should be allowed. This is because, while the Office Action focuses on the reasons why the "treatment of inflammatory or autoimmune diseases" is allegedly not enabled by the specification, no reasons as to why the subject matter recited by claims 1 and 7, i.e., the "reduction of elevated levels of PDE IV," is not patentable.

In this regard, Applicants previously submitted data showing the efficacy of various compounds encompassed by the genus recited by claim 1, including the compound specifically recited by claim 7, in reducing PDE IV levels. Indeed, the Examiners, during the personal interview held on October 9, 2007, agreed that there is little question as to whether claims 1 and 7 are enabled, in view of the previously submitted data. Consequently, while Applicants strongly believe that all of the pending claims are allowable, at least for the reasons set forth in detail below, Applicants respectfully request that, at the very least, claims 1 and 5 should be allowed as no reasons for rejecting these claims are articulated in the Office Action, and as sufficient data to show that these claims are enabled were previously submitted.

B. The Rejection Under 35 U.S.C. § 112 Should be Withdrawn

On pages 3-15 of the Office Action, the rejection of the claims as allegedly failing to comply with the enablement requirement under 35 U.S.C. § 112, first paragraph, is maintained. The bases for this allegation appear to be two-fold: 1) that the Examiner does not agree that holding and reasoning set forth in *In re Brana* are applicable in this application; and 2) that the Examiner believes that Bielekova *et al.*, *The Journal of Immunology*, 164: 1117-24 (2000) ("Bielekova")[1] provides "reason to doubt" that the claims are enabled. Each of these bases is discussed below.

(1) *In re Brana* is indeed applicable

On page 5 of the Office Action, the Examiner alleges that the holding and reasoning set forth in *In re Brana* are not application for the current application. The allegation is based on his assertion that: 1) while the issue in *Brana* was "whether 'asserted utility' was a proper test fro determining enablement under 112, first paragraph," no such issue is present in the current application (Office Action, page 5); and 2) the facts of *Brana* are distinguishable from those in the current application since *Brana*'s determination of anti-cancer activity was obtained using "a well-recognized mouse model," whereas "no *in vitro* model is well-recognized in the art for reliably and predictably determining the anti-PDE IV activity ... against a specific disease species or the genus of inflammatory and autoimmune diseases." (*Id.*). Applicants disagree with each of these allegations.

With regard to the Examiner's first allegation that the legal issue presented in *Brana* does not exist in the current application, Applicants respectfully invite the Examiner's attention to page 1564 of *Brana* decision. There, the court clearly indicated that, although the rejection at issue in *Brana* "appears to be based on the issue of whether the compounds had a practical utility, ... the rejection ... stands on the requirements of § 112, ¶ 1." (*Brana*, 51 F.3d at 1564) (emphasis added). Thus, the court stated that "it is to that provision that [the court] address" the issue. (*Id.*). In other words, the issue that the court considered and addressed in *Brana* was whether the specification had an enabling disclosure as to the asserted utility of treatment of cancer, an issue substantially identical to the one in this application, *i.e.*, whether the specification had an enabling disclosure as to the claimed utility of the treatment of inflammatory or autoimmune diseases. Therefore, Applicants respectfully point out that the rejection of the pending claims is based on the Examiner's misunderstanding and mischaracterization of the issue in *Brana*.

In addition, it is also alleged in the Office Action that facts in the current application are distinguishable from those in *Brana* allegedly because, unlike *Brana*, no well-recognized model for reliably and predictably determining the efficacy for inflammatory and autoimmune diseases is available in the art. (Office Action, page 5). Applicants respectfully, but strongly, disagree with this allegation for following several different reasons.

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[1] Initially provided by Applicants as evidence to support the enablement.

First, Applicants respectfully point out that no evidence to support the Examiner's conclusion that no art-accepted model is available for the treatment of inflammatory or autoimmune diseases. Indeed, Applicants submitted in their previous response several references, which clearly show that PDE IV is implicated with various inflammatory and/or autoimmune diseases. If such a showing is not sufficient to establish that compounds efficacious in inhibiting PDE IV would be potentially useful in treating inflammatory and/or autoimmune diseases, Applicants are at a loss as to what can satisfy this requirement.

All that is provided by the Examiner in response is a textbook excerpt which allegedly shows that "a diverse group of inflammatory and autoimmune disease" are "characterized by different immunopathologies, etiologies, and clinical manifestations." (Office Action, page 5). However, Applicants respectfully submit that cancer, which was at issue in *Brana*, also presumably encompasses various types that are "characterized by different immunopathologies, etiologies, and clinical manifestations," was found to be enabled in *Brana*. (*See Brana*, 51 F.3d at 1566).

In reaching this holding, the *Brana* court clearly indicated that "the nature of applicants' invention," *i.e.*, the treatment of cancer using chemical compounds, would not "cause one of skill in the art to reasonably doubt asserted usefulness." (*Id.*). In other words, the court's holding implies that the test in murine models against only limited types of cancer cell lines would suffice to establish that the treatment of cancer, different types of which are presumably characterized by different immunopathologies, etiologies, and clinical manifestations, was enabled. Likewise, Applicants respectfully point out that the Examiner, by merely providing that the claimed inflammatory or autoimmune diseases are complex in nature, has simply not met his burden of providing any basis that "cause one of skill in the art to reasonably doubt asserted usefulness" of the claimed treatment. (*Id.*).

For avoidance of any doubt, Applicants submit herewith the following additional references: 1) U.S. Patent No. 5,792,774, attached hereto as **Exhibit A**; 2) U.S. Patent No. 5,889, 014, attached hereto as **Exhibit B**; 3) U.S. Patent No. 5,998,428, attached hereto as **Exhibit C**; and 4) U.S. Patent No. 5,716,954, attached hereto as **Exhibit D**. As the Examiner will see, each of these U.S. patents discloses a group of chemical compounds and their asserted efficacy as PDE IV inhibitors. Based on that disclosure, each of these patents claims the use of the compounds that it discloses for the treatment of "inflammatory" and/or "autoimmune" diseases. Apart from the fact that consistency requires that the claims currently pending in this application be found allowable, these U.S. patents at least show that *in vitro* PDE IV inhibition tests are an art-recognized indicator of a compound's potential therapeutic value in treating inflammatory and/or autoimmune diseases.[2]

In sum, Applicants respectfully submit that the holding and reasoning of *Brana* are indeed applicable to this application, as the legal issue and facts considered and addressed by the court in *Brana* are substantially identical to those in this application. Consequently, to the extent that the rejection of the claims under 35 U.S.C. § 103 is based on the Examiner's misinterpretation of *Brana*, and on the unsupported allegation that the test results provided in this application are not art-recognized indicators, it is respectfully requested that the rejection be withdrawn.

(2) Bielekova does ***not*** provide any reason to doubt enablement

In the Office Action, the Examiner provides Bielekova as a reference which allegedly provides the reason to doubt that the claims are enabled. In particular, while passages from Bielekova are extensively reproduced in the Office Action, no explanation as to why the reproduced passages are relevant in determining whether there was a reason to doubt that the claims are enabled. Instead, the only suggestion that Applicants can glimpse from the Office Action is the statement from Bielekova, which is quoted in bald characters, that "**simple extrapolation of therapeutic efficacy from animal models to human disorders is not easily feasible**." (Office Action, page 7) (emphasis in original). Presumably, Applicants believe that it is the Examiner's position that there was reason to doubt the enablement because Bielekova states that therapeutic efficacy obtained from animal (or any other models, including the *in vitro* tests provided in this application) cannot easily be extrapolated to human disorders.

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[2] It is noted that the Examiner, relying on Bielekova, alleges that the prediction of the ultimate value in human therapy based on the PDE IV inhibition model cannot be made. Even if taken true, such an allegation is completely irrelevant to the patentability of the pending claims, as discussed in more detail below.

Regardless of the truth of that statement, Applicants respectfully point out that it is legally erroneous to rely on such a statement in rejecting the claims for allegedly failing to satisfy the enablement requirement. This is because the court in *Brana* considered a substantially identical disclosure made in a prior art reference (*i.e.*, disclosure in a prior art reference that some laboratory oncologists are skeptical about the predictive value of murine tumor models provided by the applicants for human therapy), and concluded that the claims were enabled because the "usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development." (*Brana*, 51 F.3d at 1568). In reaching this conclusion, the court held that the references that "merely discuss the therapeutic predictive value of [the tests provided by the applicants]" are relevant in assessing the enablement requirement "only if applicants must prove the ultimate value in humans of their asserted utility," but held that no such requirement exists for the patentability. (*Id.* at 1566) (emphasis added).

Applying the holdings of *Brana* to the current application, Bielekova, especially the portion relied on by the Examiner in Bielekova, precisely discloses what the prior art references at issue in *Brana* disclosed (*i.e.*, that the predictive value of certain non-human tests in human therapy may be questionable). However, as the *Brana* court plainly puts it, such a disclosure is relevant "only if applicants must prove the ultimate value in humans of their asserted utility." (*Id.*). Further, as the *Brana* court plainly puts it, Applicants need not prove the ultimate value in human therapy. This is because, according to the *Brana* court, "[t]he stage at which an invention in [the pharmaceutical] field becomes useful is well before it is ready to be administered to humans." (*Id.* at 1568).

Consequently, it is clear that the Examiner, by relying on the disclosure in Bielekova, has not provided any reasons to doubt that the pending claims are enabled. Further, to the extent that the holdings in *Brana* may require that the enablement requirement be satisfied by providing an art-recognized test that can be an indication of the claimed efficacy[3], such a requirement has been satisfied by Applicants' submission of the test results, along with various references that show the linkage between PDE IV inhibition and the treatment of inflammatory and/or autoimmune diseases. Therefore, Applicants respectfully submit that the pending claims are enabled and comply with all of the requirements of 35 U.S.C. § 112, first paragraph. Thus, reconsideration and withdrawal of this ground of rejection is respectfully requested.

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[3] Applicants, however, respectfully point out that no burden lies on Applicants to provide such evidence, either. This is because the Examiner failed to meet his initial burden of producing evidence that provides reasons to doubt the enablement. Nevertheless, Applicants respectfully point out that even assuming the Examiner's initial burden has been met, Applicants, by providing the art-recognized PDE IV inhibition test results, have satisfied any burden whatsoever regarding the enablement requirement.

C. The Rejection Under Obviousness-Type Double Patenting Should Be Withdrawn

On pages 15-16 of the Office Action, the pending claims are rejected under the judicially-created obviousness-type double patenting over claims 47-70 of the U.S. Application No. 10/786,822 ("the '822 application")[4]. Applicants respectfully traverse this rejection.

Specifically, Applicants respectfully point out that the genus structures recited by claims 47-70 of the '822 application do not overlap with the genus structure recited by the pending claims. In particular, it is pointed out that the R^3 position of the currently recited genus cannot be $-CO-(NR^4)-O-CO-R^1$ as required by the genus structures recited by claims 47-70 of the '822 application. Therefore, in view of these structural differences, the pending claims cannot be obvious over claims 47-70 of the '822 application, and no reasoning or evidence to the contrary is provided in the Office Action. Thus, Applicants respectfully request that this rejection be withdrawn.

D. Conclusion

For at least the foregoing reasons, Applicants respectfully submit that all of the pending claims are allowable, and thus, respectfully request that all of the rejections be withdrawn.

In particular, Applicants respectfully submit that: 1) the pending enablement rejection cannot stand in view of the holding and reasoning provided in *In re Brana*; 2) sufficient evidence to show that the PDE IV test results provided by Applicants are indeed well-recognized in the art as an indicator of therapeutic efficacy in treating inflammatory and/or autoimmune diseases was previously and is currently provided by Applicants; and 3) the Examiner's reliance on Bielekova as a reference that provides reason to doubt the enablement is misplaced.

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[4] Applicants respectfully point out that the '822 application recently issued as U.S. Patent No. 7,345,062, without claims 47-70. However, a continuation application containing claims 47-70 have been filed from the '822 application, and was accorded a serial number 12/009,182.

Finally, Applicants respectfully submit that, at the very least, claims 1 and 7 should be found allowable because it is clear that the PDE IV inhibition data provided by Applicants would enable the claims to "reduction of PDE IV," as recited by claims 1 and 7, and because no reasoning or basis for other rejection is provided in connection with claims 1 and 7 by the Examiner.

No fee is believed due for the submission of this paper. However, if any fees are required for the submission of this paper, or to avoid abandonment of this application, the Director is authorized to charge such fees to Jones Day Deposit Account No. 503013.

Respectfully Submitted,

Date: March 25, 2008

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Exhibit A

US005792774A

United States Patent [19]

Haughan et al.

[11] **Patent Number: 5,792,774**

[45] **Date of Patent: Aug. 11, 1998**

[54] QUINOLONES AND THEIR THERAPEUTIC USE

[75] Inventors: **Alan Findlay Haughan; Steven Colin Beasley; John Gary Montana; Robert John Watson**, all of Cambridge, United Kingdom

[73] Assignee: **Chiroscience Limited**, Cambridge, United Kingdom

[21] Appl. No.: **803,731**

[22] Filed: **Feb. 21, 1997**

[30] **Foreign Application Priority Data**

Feb. 21, 1996	[GB]	United Kingdom	9603654
Feb. 21, 1996	[GB]	United Kingdom	9603655
Feb. 21, 1996	[GB]	United Kingdom	9603689

[51] **Int. Cl.**^{6} **A61K 31/435**; C07D 221/06

[52] **U.S. Cl.** **514/294**; 546/94

[58] **Field of Search** 546/94, 95; 514/294

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,001,243	1/1977	Gerster	260/287 P
4,317,820	3/1982	Ishikawa et al.	424/246
4,416,884	11/1983	Ishikawa et al.	424/250

FOREIGN PATENT DOCUMENTS

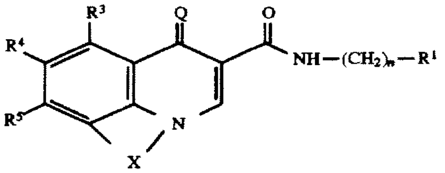
1433151	4/1976	United Kingdom .
2236751	4/1991	United Kingdom .

Primary Examiner—Joseph McKane

Attorney, Agent, or Firm—Saliwanchik, Lloyd & Saliwanchik

[57] **ABSTRACT**

A compound of formula (I)



(I)

or a pharmaceutically-acceptable salt, solvate or hydrate thereof.

23 Claims, No Drawings

QUINOLONES AND THEIR THERAPEUTIC USE

FIELD OF THE INVENTION

The present invention relates to novel quinolone derivatives and pharmaceutically-acceptable salts thereof, processes for their production, and their formulation and use as pharmaceuticals.

BACKGROUND OF THE INVENTION

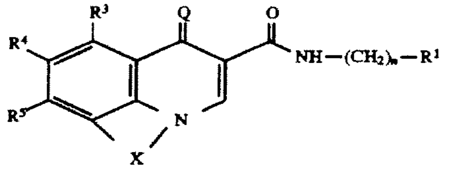
Quinolone and quinolizine compounds are known mainly as antibacterial agents (JP-A-05025162; U.S. Pat. No. 5,037,834; EP-A-0420069; JP-A-02040379; EP-A-0343560; DE-A-3816119; EP-A-0304158; FR-A-2644784; WO-A-9410163; DE-A-3641312) or antiviral agents (U.S. Pat. No. 4,959,363), but also as inhibitors of 5-lipoxygenase (JP-A-02124871), cardiotonics and vasodilators (JP-A-01061461) and 5-HT_3 antagonists for the treatment of peripheral disorders associated with pain (WO-A-9501793; GB-A-2236751).

GB-A-2236751 describes quinolone-3-carboxamides as 5-HT_3 antagonists, for use in the treatment of neuropsychiatric disorders. U.S. Pat. No. 4,001,243 discloses benzoquinoline-2-carboxylic acids and esters as antimicrobial agents. GB-A-1433151 discloses N-tetrazolylbenzoquinolizine-2-carboxamides, as agents that may be useful in the treatment of allergies including asthma.

Phosphodiesterases (PDE) and Tumour Necrosis Factor (TNF), their modes of action and the therapeutic utilities of inhibitors thereof, are described in WO-A-9704775 and WO-A-9704779, the contents of which are incorporated herein by reference. The same documents disclose quinolones having utility as PDE and TNF inhibitors.

SUMMARY OF THE INVENTION

This invention relates to compounds and their utility to treat disease states, for example disease states associated with proteins that mediate cellular activity, for example by inhibiting tumour necrosis factor and/or by inhibiting phosphodiesterase IV. According to the invention, novel compounds are of formula (I):



(I)

wherein n is 1, 2 or 3;

R^1 represents cycloalkl, aryl, heteroaryl or heterocyclo, in which any ring may be fused to a second ring selected from aryl and heteroaryl, to give a bicyclic structure, and in which any group is optionally substituted by one or more substituents chosen from halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H (or C_{1-6} alkyl esters or C_{1-6} alkyl amides thereof), C_{1-6} alkyl, NR^6R^7 and $SO_2NR^6R^7$;

R^3, R^4 and R^5 are the same or different and represent H, halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H (or C_{1-6} alkyl esters or C_{1-6} alkyl amides thereof), NR^6R^7, $SO_2NR^6R^7$ or C_{1-6} alkyl in which alkyl is optionally substituted with halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H (or C_{1-6} alkyl esters or C_{1-6} alkyl amides thereof), NR^6R^7 or $SO_2NR^6R^7$, or any adjacent two substituents R^3–R^5 are joined to form an optionally substituted carbocyclic aromatic, heteroaromatic, saturated carbocyclic or heterocyclic ring;

R^6 and R^7 are the same or different and represent H, C_{1-6} alkyl, cycloalkyl, C_{1-6} alkylcarbonyl, arylcarbonyl, C_{1-6} alkoxycarbonyl, arylsulphonyl or C_{1-6} alkylsulphonyl or NR^6R^7 is a 5 or 6-membered ring such as pyrrolidine, piperidine, morpholine or piperazine;

X represents a linking group selected from $—(CR^9R^{10})_{2-3}—$, $—Y—(CR^9R^{10})_2—$, $—(CR^9R^{10})_2—Y—$, $—CR^9R^{10}—Y—CR^9R^{10}—$ and $—Y—CR^9R^{10}—Z—$, Y and Z being independently NR^{11}, O or $S(O)_{0-2}$, provided that Y and Z are not both $S(O)_{0-2}$;

Q represents O or S; and

each R^9, each R^{10} and R^{11} are the same or different and are H or C_{1-6} alkyl;

and pharmaceutically-acceptable salts, solvates and hydrates thereof

Combinations of substituents and/or variables are only permissible if such combinations result in stable compounds.

DESCRIPTION OF THE INVENTION

Suitable pharmaceutically-acceptable salts are pharmaceutically-acceptable base salts and pharmaceutically-acceptable acid addition salts. Certain of the compounds of formula (I) which contain an acidic group form base salts. Suitable pharmaceutically-acceptable base salts include metal salts, such as alkali metal salts for example sodium salts, or organic amine salts such as that provided with ethylenediamine.

Certain of the compounds of formula (I) which contain an amino group form acid addition salts. Suitable acid addition salts include pharmaceutically-acceptable inorganic salts such as the sulphate, nitrate, phosphate, borate, hydrochloride and hydrobromide and pharmaceutically-acceptable organic acid addition salts such as acetate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methanesulphate, α-ketoglutarate, α-glycerophosphate and glucose-1-phosphate. The pharmaceutically-acceptable salts of the compounds of formula (I) are prepared using conventional procedures.

It will be appreciated by those skilled in the art that some compounds of formula (I) can exist in more than one tautometric form. This invention extends to all tautomeric forms.

It will be appreciated that some of the compounds according to the invention can contain one or more asymmetrically-substituted carbon and/or sulphur atoms. The presence of one or more of these asymmetric centers in a compound of formula (I) can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers, and diastereoisomers and mixtures, including racemic mixtures, thereof.

When used herein the term alkyl whether used alone or when used as part of another group includes straight and branched chain alkyl groups. Cycloalkyl includes a non-aromatic cyclic or multicyclic ring system of about 3 to 10 carbon atoms. The cyclic alkyl may optionally be partially unsaturated. Alkoxy means an alkyl-O-group in which the alkyl group is as previously described. Alkyl amide includes both monoalkyl and dialkyl amides, in which the alkyl groups (previously defined) may be the same or different. Alkylcarbonyl means an alkyl-CO-group in which the alkyl group is as previously described. Aryl indicates carbocyclic radicals containing about 6 to 10 carbon atoms. Heteroaryl means an about 5 to about 10-membered aromatic monocyclic or multicyclic ring system in which one or more of the atoms in the ring system is an element other than carbon, chosen from amongst nitrogen, oxygen or sulphur. Heterocyclo means an about 5 to about 10-membered saturated or partially saturated monocyclic or multicyclic ring system in which one or more of the atoms in the ring system is an element other than carbon, chosen from amongst nitrogen, oxygen or sulphur. Arylcarbonyl means an aryl-CO-group. Arylsulphonyl means an aryl-SO_2- group. Alkylsulphonyl means an alkyl-SO_2-group. Alkoxycarbonyl means an alkoxy-CO-group. When any two of R^3, R^4 and R^5 are joined together, an example is methylenedioxy (OCH_2O). Halo means fluorine, chlorine, bromine or iodine.

The compounds of formula (I) are preferably in pharmaceutically-acceptable form. By pharmaceutically-acceptable form is meant, inter alia, a pharmaceutically-acceptable level of purity excluding normal pharmaceutical additives such as diluents and carriers, and including no material considered toxic at normal dosage levels. A pharmaceutically-acceptable level of purity will generally be at least 50% excluding normal pharmaceutical additives, preferably 75%, more preferably 90% and still more preferably 95%.

Compounds of the invention can be used in the prevention or treatment of "TNF-mediated disease or disease states", i.e. any and all disease states in which TNF plays a role, either by production of TNF itself, or by TNF causing another cytokine to be released, such as but not limited to IL-1 or IL-6. A disease state in which IL-1, for instance, is a major component, and whose production or action is exacerbated or secreted in response to TNF, would therefore be considered a disease state mediated by TNF. As TNF-β (also known as lymphotoxin) has close structural homology with TNF-α (also known as cachectin), and since each induces similar biologic responses and binds to the same cellular receptor, both TNF-α and TNF-β are considered to be inhibited by compounds of the present invention and thus are herein referred to collectively as "TNF" unless specifically indicated otherwise.

This invention relates to a method for mediating or inhibiting the enzymatic activity or catalytic activity of PDE IV in a mammal in need thereof and for inhibiting the production of TNF in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound of Formula (I) or a pharmaceutically-acceptable salt thereof.

PDE IV inhibitors are useful in the treatment of a variety of allergic and inflammatory diseases, including: asthma, chronic bronchitis, atopic dermatitis, urticaria, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, inflammation of the eye, allergic responses in the eye, eosinophilic granuloma, psoriasis, Bechet's disease, erythematosis, anaphylactoid purpura nephritis, joint inflammation, arthritis, rheumatoid arthritis and other arthritic conditions such as rheumatoid spondylitis and osteoarthritis, septic shock, ulcerative colitis, Crohn's disease, reperfusion injury of the myocardium and brain, chronic glomerulonephritis, endotoxic shock and adult respiratory distress syndrome. In addition, PDE IV inhibitors are useful in the treatment of diabetes insipidus and conditions associated with cerebral metabolic inhibition, such as cerebral senility, senile dementia (Alzheimer's disease), memory impairment associated with Parkinson's disease, depression and multi-infarct dementia PDE IV inhibitors are also useful in conditions ameliorated by neuroprotectant activity, such as cardiac arrest, stroke and intermittent claudication. PDE IV inhibitors may be useful in the treatment of tardive dyskinesia, ischaemia and Huntingdon's disease. Additionally, PDE IV inhibitors could have utility as gastroprotectants. A special embodiment of the therapeutic methods of the present invention is the treatment of asthma.

The viruses contemplated for treatment herein are those that produce TNF as a result of infection, or those which are sensitive to inhibition, such as by decreased replication, directly or indirectly, by the TNF inhibitors of Formula (I). Such viruses include, but are not limited to HIV-1, HIV-2 and HIV-3, cytomegalovirus (CMV), influenza, adenovirus and the Herpes group of viruses, such as, but not limited to, *Herpes zoster* and *Herpes simplex*.

This invention more specifically relates to a method of treating a mammal, afflicted with a human immunodeficiency virus (HV), which comprises administering to such mammal an effective TNF-inhibiting amount of a compound of Formula (I) or a pharmaceutically-acceptable salt thereof.

The compounds of this invention may be also be used in association with the veterinary treatment of animals, other than humans, in need of inhibition of TNF production. TNF mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples of such viruses include, but are not limited to, feline immunodeficiency virus (FIV) and other retroviral infections such as equine infectious anaemia virus, caprine arthritis virus, visna virus, maedi virus and other lentiviruses.

The compounds of this invention are also useful in treating parasite, yeast and fungal infections, where such yeast and fungi are sensitive to upregulation by TNF or will elicit TNF production in vivo. A preferred disease state for treatment is fungal meningitis.

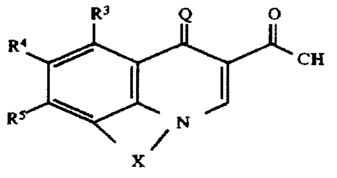
Compounds of the invention may also suppress neurogenic inflammation through elevation of cAMP in sensory neurones. They are, therefore, analgesic, anti-tussive and anti-hyperalgesic in inflammatory disease associated with irritation and pain.

Compounds of the general formula (I) may be prepared by any suitable method known in the art and/or by the following process, which itself forms part of the invention.

In the description and formulae below the groups R^1, R^3, R^4, R^5, R^6, R^7, R^9, R^{10}, R^{11}, Q, X and Z are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds described below, and which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable protecting groups for such functionalities will be apparent to those skilled in the art. For specific details see "Protective Groups in Organic Synthesis", Wiley Interscience, T W Greene, PGM Wuts. Thus a process for preparing compounds of formula (I) in which, say, R^4 is CO_2H comprises deprotecting (for example by hydrolysis) a compound of formula (I) in which R^4 is CO_2R wherein R represents a suitable protecting group (e.g. methyl).

A preferred embodiment of formula (I) comprises those compounds wherein X is $(CR^9R^{10})_3$.

A process for preparing compounds of general formula (I) comprises coupling an acid of formula (II)



(II)

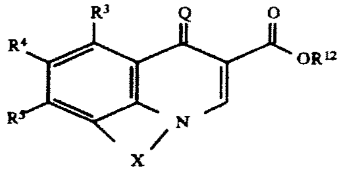
or an activated derivative thereof, with an amine of formula (III)

$NH_2—(CH_2)_n—R^1$ (III)

Amines of formula (III) are commercially available or can be readily obtained from commercially-available starting materials using methods known to those skilled in the art. Some of the amines of formula (III) are conveniently prepared by reductive amination of an appropriate carbonyl compound with a suitable amine. This amination may be carried out under any suitable standard conditions known to those skilled in the art. Active derivatives of acids of formula (II) include, for example, acid anhydrides and acid halides, such as acid chlorides.

The coupling reaction may be performed using standard conditions for amination reactions of this type. Thus, the reaction may be achieved in a solvent, for example an inert organic solvent such as an ether, e.g. a cyclic ether such as tetrahydrofuran, an amide, e.g. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane, at a low temperature, e.g. −30° C. to ambient temperature, such as −20° C. to 0° C., optionally in the presence of a base, e.g. an organic base such as an amine, e.g. triethylamine or a cyclic amine such as N-methylmorpholine. The reaction may additionally be performed in the presence of a condensing agent, for example a diimide such as N,N-dicyclohexylcarbodiimide, advantageously in the presence of a triazole such as 1-hydroxybenzotriazole. Alternatively, the acid may be reacted with a chloroformate, for example ethyl chloroformate, prior to reaction with the amine of formula (III).

Acids of general formula (II) may be prepared according to the procedure described in EP-A-0373531, DE-A-0683169 or by Kaminsky and Meltzer, *J. Med. Chem.*, 11:160–164 (1968). This procedure includes hydrolysis of the corresponding ester of general formula (IV)

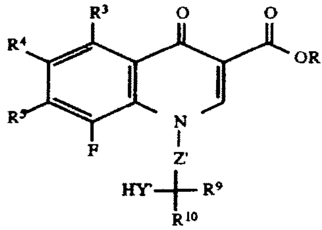


(IV)

where R^{12} represents an (ar)alkyl group such as methyl, ethyl, benzyl or tert-butyl.

Compounds of formula (IV) in which Q=S may be derived from the corresponding compounds where Q=O using standard conditions for sulphurisation of such compounds. For example, suitable conditions comprise reaction with phosphorus pentasulphide (P_4S_{10}) in an organic solvent such as pyridine at an appropriate temperature. The reflux temperature of the solvent is preferred.

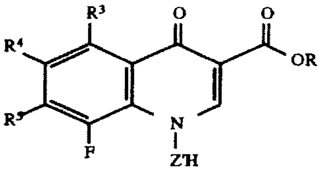
Esters of general formula IV wherein X is —Y'—CR^9R^{10}—Z', Y' and Z' each being NR^{11}, O or S, e.g. —O—CR^9R^{10}—NR^{11}—, may be prepared by cyclisation of intermediates of general formula (V)



(V)

using procedures evident to those skilled in the art; for example, cyclisation may utilise a tetraalkylammonium fluoride such as tetrabutylammonium fluoride as reagent. Y' or Z' as S may subsequently be converted to SO or SO_2.

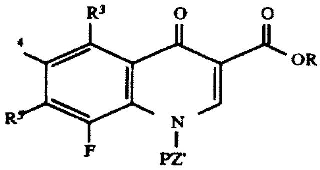
Intermediates of general formula (V) may be prepared by formylation of a corresponding amine or other intermediate of general formula (VI)



(VI)

using, for example, formaldehyde.

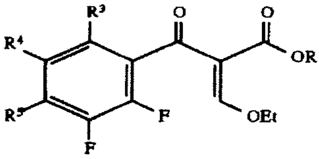
Compounds of general formula (VI) may be prepared from the corresponding protected compounds of general formula (VII)



(VII)

wherein P is any suitable protecting group, e.g. Boc, by deprotection using methods evident to those skilled in the art, such as reaction with base.

The quinoline nucleus in the above compounds may be generated by reaction of an ester of formula (VIII)

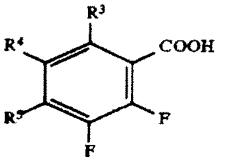


(VIII)

with an amine of general formula (IX)

$H_2N—Z'P$ (IX)

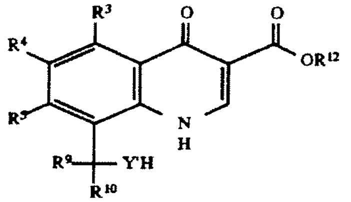
Intermediates of general formula (VIII) may be made from benzoic acids of general formula (X)



(X)

using methodology analogous to that reported previously (J. Med. Chem. 34:1142 (1991)).

When X is —CR^9R^{10}—Y'—CR^9R^{10}—, esters of general formula (IV) may be prepared from the corresponding intermediate of general formula (XI)

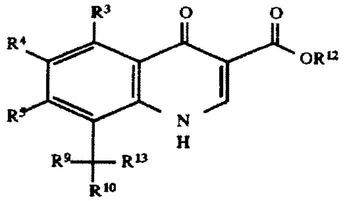


(XI)

by reaction with a formate derivative of general formula (XII)

$R^9R^{10}C(OR^{12})_2$ (XII)

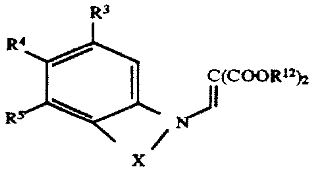
Compounds of general formula (XI) may be prepared by hydrolysis of the corresponding intermediates (XIII)



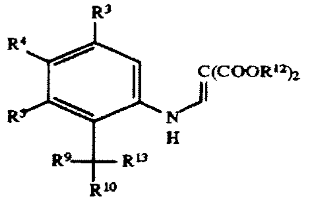
(XIII)

wherein R^{13} represents a protected form of YH such as OAc, SAc or $NR^{11}Boc$.

The quinoline nucleus in the compounds (IV) and (XIII) may be generated by cyclisation of an ester of formula (XIV) or (XV)



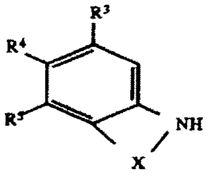
(XIV)



(XV)

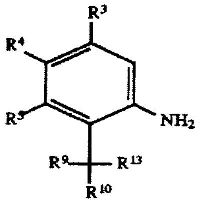
using methodology analogous to that described by Kaminsky and Meltzer, supra. Suitable conditions include, for example, heating to reflux in diphenyl ether or a eutectic mixture of diphenyl ether and biphenyl.

Compounds of formula (XIV) or (XV) may be prepared by the reaction of an aniline of general formula (XVI) or (XVII)



(XVI)

-continued



(XVII)

with a dialkl alkoxyethylidinemalonate of the formula (XVIII)

$(R^{12}OOC)_2C{=}CH{-}OR^{14}$ (XVIII)

wherein R^{14} is a lower alkyl group such as methyl or ethyl. This reaction may be carried out under suitable standard conditions known to those skilled in the art, for example those described by Kaminsky and Meltzer, supra. For example, the reaction may be carried out at elevated temperature, for example 80°–150° C., in an inert solvent (such as xylene) or, preferably, in the absence of solvent.

Intermediates of formulae (IX), (X), (XII), (XVI), (XVII) and (XVIII) are commercially available or can be readily obtained from commercially available starting materials using methods known to those skilled in the art.

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R^4 is C_{1-6} alkoxy may be prepared by appropriate alkylation of a compound of formula (I) wherein R^4 is a hydroxy group.

Any mixtures of final products or intermediates obtained can be separated on the basis of the physico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under the circumstances. It will be appreciated that where a particular stereoisomer of formula (I) is required, this may be obtained by conventional resolution techniques such as high performance liquid chromatography. Where desired, however, appropriate homochiral starting materials may be used in the reaction sequence to yield a particular stereoisomer of formula (I).

A compound of formula (I) or where appropriate a pharmaceutically-acceptable salt thereof and/or a pharmaceutically-acceptable solvate thereof, may be administered per se or, preferably, as a pharmaceutical composition also comprising a pharmaceutically-acceptable carrier.

Accordingly, the present invention provides a pharmaceutical composition comprising a compound of formula (I) or where appropriate a pharmaceutically-acceptable salt thereof and/or a pharmaceutically-acceptable solvate thereof, and a pharmaceutically-acceptable carrier.

The active compound may be formulated for administration by any suitable route, the preferred route depending upon the disorder for which treatment is required, and is preferably in unit dosage form or in a form that a human patient may administer to himself in a single dosage. Advantageously, the composition is suitable for oral, rectal, topical, parenteral administration or through the respiratory tract. Preparations may be designed to give slow release of the active ingredient.

The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle, sheep, dogs, cats, etc, the compounds of the invention are effective in the treatment of humans.

The compositions of the invention may be in the form of tablets, capsules, sachets, vials, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations such as oral or sterile parenteral solutions or suspensions. Topical formulations are also envisaged where appropriate.

In order to obtain consistency of administration it is preferred that a composition of the invention is in the form of a unit dose. Unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycolate or microcrystalline cellulose; or pharmaceutically-acceptable wetting agents such as sodium lauryl sulphate.

Solid oral compositions may be prepared by conventional methods of blending, filling, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers.

Such operations are of course conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of, for example, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and, as desired, conventional flavouring or colouring agents.

Compositions may also suitably be presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebuliser, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less than 50 μm, such as from 0.1 to 50 μm, preferably less than 10 μm, for example from 1 to 10 μm, 1 to 5 μm or from 2 to 5 μm. Where appropriate, small amounts of other anti-asthmatics and bronchodilators for example sympathomimetic amines such as isoprenaline, isoetharine, salbutamol, phenylephrine and ephedrine; corticosteroids such as prednisolone and adrenal stimulants such as ACTH may be included.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, and, depending on the concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, adjuvants such as local anaesthetic, a preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially

the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilisation cannot be accomplished by filtration. The compound can be sterilised by exposure to a suitable sterilising agent, before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% to 99% by weight, preferably from 10–60% by weight, of the active material, depending on the method of administration.

Compounds of formula (I), or if appropriate a pharmaceutically-acceptable salt thereof and/or a pharmaceutically-acceptable solvate thereof, may also be administered as a topical formulation in combination with conventional topical excipients.

Topical formulations may be presented as, for instance, ointments, creams or lotions, impregnated dressings, gels, gel sticks, spray and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams. The formulations may contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions.

Suitable cream, lotion, gel, stick, ointment, spray or aerosol formulations that may be used for compounds of formula (I) or if appropriate a pharmaceutically-acceptable salt thereon are conventional formulations well known in the art, for example, as described in standard text books such as Harry's Cosmeticology published by Leonard Hill Books, Remington's Pharmaceutical Sciences, and the British and US Pharmacopoeias.

Suitably, the compound of formula (I), or if appropriate a pharmaceutically-acceptable salt thereof, will comprise from about 0.5 to 20% by weight of the formulation, preferably from about 1 to 10%, for example 2 to 5%.

The dose of the compound used in the treatment of the invention may be determined by the skilled man, and will vary in the usual way with the seriousness of the disorders, the weight of the sufferer, and the relative efficacy of the compound. However, as a general guide suitable unit doses may be 0.1 to 1000 mg, such as 0.5 to 200, 0.5 to 100 or 0.5 to 10 mg, for example 0.5, 1, 2, 3, 4 or 5 mg; and such unit doses may be administered more than once a day, for example 2, 3, 4, 5 or 6 times a day, but preferably 1 or 2 times per day, so that the total daily dosage for a 70 kg adult is in the range of about 0.1 to 1000 mg, that is in the range of about 0.001 to 20 mg/kday, such as 0.007 to 3, 0.007 to 1.4, 0.007 to 0.14 or 0.01 to 0.5 mg/kg/day, for example 0.01, 0.02, 0.04, 0.05, 0.06, 0.08, 0.1 or 0.2 mg(kg/day, and such therapy may extend for a number of weeks or months.

When used herein the term "pharmaceutically-acceptable" encompasses materials suitable for both human and veterinary use. No toxicological effects have been established for compounds of formula (I) in the above-mentioned dosage ranges.

The following Examples illustrate the invention.

EXAMPLE 1

9-fluoro-6,7-dihydro-5-methyl-N-[2-(4-pyridyl)ethyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamide

Flumequine (0.46 g) and dichloromethane (16.3 ml) were combined under nitrogen and cooled to 0° C. Triethylamnine (0.27 ml) was then added dropwise, followed by isopropenyl chloroformate (0.21 ml) and the whole stirred for 60 minutes. 4-(2-Aminoethyl)pyridine (0.23 ml) was then added and the reaction stirred for 20 h, concentrated onto silica and purified by flash column chromatography to give the title compound (0.55 g) as a white solid.

TLC R_f=0.27 (5% MeOH/CH_2Cl_2) mp=217° C.

EXAMPLE 2

9-fluoro-6,7-dihydro-5-methyl-N-[2-(4-pyridyl)ethyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamide hydrochloride

The product of Example 1 (393.2 mg) and chloroform (4 ml) were combined and stirred at room temperature. A solution of 1M hydrochloric acid in ether (1.08 ml) was then added and the resulting precipitate filtered and dried to give the title compound (433.4 mg) as a white solid. m.p.=268°–269° C.

^{1}HNMR (DMSO): 1.35(t, 3H), 2.1(m, 2H), 3.1(m, 4H), 3.7(m, 2H), 4.8(m, 1H), 7.6–8.8 (7H, aromatic), 10.0(t,1H).

EXAMPLE 3

9-fluoro-6,7-dihydro-5-methyl-N-[(4-pyridyl)methyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamide

Flumequine (0.46 g) and dichloromethane (16.3 ml) were combined under nitrogen and cooled to 0° C. Triethylamine (0.27 ml) was then added dropwise, followed by isopropenylchloroformate (0.21 ml) and the whole stirred for 60 minutes. 4-(Aminomethyl)pyridine (0.2 ml) was then added and the reaction stirred for 20 h, after which time it was diluted with dichloromethane, washed with water (3 times), dried ($MgSO_4$), concentrated in vacuo and triturated with acetone to give the title compound (0.49 g) as a yellow solid.

TLC R_f=0.43 (10% MeOH/CH_2Cl_2) m.p.=199°–200° C.

EXAMPLE 4

9-fluoro-6,7-dihydro-5-methyl-N-[(4-pyridyl)methyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamchloride hydrochloride

Example 4 was conducted in a similar manner to Example 2. Thus 0.12 g of the product of Example 3 and 0.35 ml of a 1M solution of HCl in ether gave the title compound as an off-white solid. m.p.=251°–253° C.

1H NMR (DMSO): 1.35(t,3H), 2.1(m,2H), 3.2(m,2H), 4.8(m, 3H), 7.6–8.8(7H aromatic), 10.6(t,1H).

Assay Methods

PDE IV Inhibition Assay

The methods used to confirm the phosphodiesterase IV inhibitory activity of formula (I) are standard assay procedures, as disclosed by Schilling et al, An. Biochem. 216:154 (1994), Thompson and Strada, Adv. Cycl. Nucl. Res. 8:119 (1979), and Gristwood and Owen, Br. J. Pharmacol. 87:91P (1985). Compounds of formula (I) have exhibited activity at levels consistent with those believed to be useful in treating phosphodiesterase IV related disease states in those assays.

TNF Inhibition Assay

The ability of the compounds of formula (I) to inhibit TNF production in human monocytes is measured as follows. Peripheral blood mononuclear cells are prepared from freshly taken blood or "Buffy coats" by standard procedures. Cells are plated out in RPMI+1% foetal calf serum in the presence and absence of inhibitors. LPS (100 ng/ml) is added and cultures are incubated for 22 h at 37° C. in an atmosphere of 95% air/5% CO_2. Supernatants are tested for TNFα by ELISA using commercially available kits.

Skin Eosinophilia Model

In vivo activity in a skin eosinophilia model is determined by using the methods described by Hellewell et al Br. J. Pharmacol. 111:811 (1994) and Br. J. Pharmacol. 110:416 (1993). Activity in a lung model is measured using the procedures described by Kallos and Kallos Int. Archs. Allergy Appl. Immunol. 73:77 (1984) and Sanjar et al Br. J. Pharmacol. 99:679 (1990).

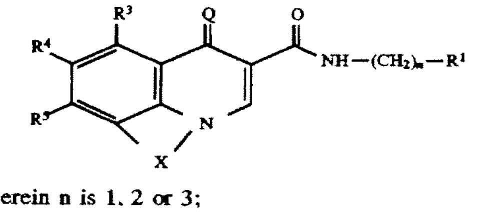
The following abbreviations have been used:

TNF tumour necrosis factor
LPS lipopolysaccharide (endotoxin)
ELISA enzyme linked immunosorbent assay

Certain general description, and description relating to the preparation of intermediates, may be found in a copending Application, for the same assignee, having the same filing date.

We claim:

1. A compound of formula (I)



(I)

wherein n is 1, 2 or 3;

R^1 is selected from the group consisting of cycloalkyl, aryl, heteroaryl, and heterocyclo, any of which rings may be fused to a second ring selected from aryl, heteroaryl, to give a bicyclic structure, and in which the or each ring is optionally substituted by one or more substituents chosen from halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H, C_{1-6} alkyl esters, C_{1-6} alkyl amides, C_{1-6} alkyl, NR^6R^7, and $SO_2NR^6R^7$;

R^3, R^4, and R^5 are the same or different and are selected from the group consisting of H, halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H, C_{1-6} alkyl esters, C_{1-6} alkyl amides, NR^6R^7, $SO_2NR^6R^7$ and C_{1-6} alkyl in which alkyl is optionally substituted with halo, C_{1-6} alkoxy, hydroxy, CN, CO_2H, C_{1-6} alkyl esters, C_{1-6} alkyl, NR^6R^7 or $SO_2NR^6R^7$, or any adjacent two substituents R^3–R^5 are joined to form an optionally substituted carbocyclic aromatic, heteroaromatic, saturated carbocyclic or heterocyclic ring;

R^6 and R^7 are the same or different and are selected from the group consisting of H, C_{1-6} alkyl, cycloalkyl, C_{1-6} alkylcarbonyl, arylcarbonyl, C_{1-6} alkoxycarbonyl, arylsulphonyl, and C_{1-6} alkylsulphonyl, or NR^6R^7 is a 5- or 6-membered ring selected from the group consisting of pyrrolidine, piperidine, morpholine, or piperazine;

X represents a linking group selected from the group consisting of $-(CR^9R^{10})_{2-3}-$, $-Y-(CR^9R^{10})_2-$, $-(CR^9R^{10})_2-Y-$, $-CR^9R^{10}-Y-CR^9R^{10}-$, and $-Y-CR^9R^{10}-Z-$, Y and Z being independently selected from the group consisting of NR^{11}, O, and $S(O)_{0-2}$, provided that Y and Z are not both $S(O)_{0-2}$;

Q represents O or S;

each R^9, each R^{10} and R^{11} are the same or different and are H or C_{1-6} alkyl;

or a pharmaceutically-acceptable salt, solvate, or hydrate thereof.

2. The compound, according to claim 1, wherein X is $-Y-CR^9R^{10}-Z-$.

3. The compound, according to claim 1, wherein X is $-CR^9R^{10}-Y-CR^9R^{10}-$.

4. The compound, according to claim 1, wherein X is selected from the group consisting of $(CR^9R^{10})_q$, $CR^9R^{10}CR^9R^{10}NR^{11}$, $NR^{11}CR^9R^{10}CR^9R^{10}$, $CR^9R^{10}CR^9R^{10}O$, $OCR^9R^{10}CR^9R^{10}$, $CR^9R^{10}CR^9R^{10}S(O)_t$, and $S(O)_tR^9R^{10}CR^9R^{10}$, q=2–3 and t=0–2.

5. The compound, according to claim 4, wherein X is $(CR^9R^{10})_3$.

6. The compound, according to claim 1, wherein Q is O.

7. The compound, according to claim 1, selected from the group consisting of

9-fluoro-6,7-dihydro-5-methyl-N-[2-(4-(2-pyridyl)ethyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamide and its hydrochloride, and

9-fluoro-6,7-dihydro-5-methyl-N-[(4-pyridyl)methyl]-1-oxo-1H,5H-benzo-[i,j]quinolizine-2-carboxamide and its hydrochloride.

8. The compound, according to claim 1, which has one or more chiral centres and is in the form of an enantiomer or diastereomer.

9. A pharmaceutical composition comprising a compound of claim 1, as active ingredient, in combination with a suitable excipient.

10. A method for treating a human or animal hosting a disease state capable of being modulated by inhibiting phosphodiesterase IV which comprises admninistering to said human or animal a disease-inhibiting amount of a compound of claim 1 in association with a pharmaceutically-acceptable carrier.

11. The method, according to claim 10, wherein the disease state is a pathological condition associated with a function of phosphodiesterase IV, eosinophil accumulation, or a function of eosinophil.

12. The method, according to claim 11, wherein the pathological condition is selected from the group consisting of asthma, chronic bronchitis, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, inflammation of the eye, allergic responses in the eye, eosinophilic granuloma, psoriasis, rheumatoid arthritis, gouty arthritis and other arthritic conditions, ulcerative colitis, Crohn's disease, adult respiratory distress syndrome, diabetes insipidus, keratosis, atopic eczema, atopic dermatitis, cerebral senility, multi-infarct dementia, senile dementia, memory impairment associated with Parkinson's disease, depression, cardiac arrest, stroke, and intermittent claudication.

13. The method, according to claim 11, wherein the pathological condition is selected from the group consisting of chronic bronchitis, allergic rhinitis, and adult respiratory distress syndrome.

14. The method, according to claim 10, wherein the disease state is capable of being modulated by TNF inhibition.

15. The method, according to claim 14, wherein the disease state is an inflammatory disease or autoimmune disease.

16. The method, according to claim 15, wherein the disease state is selected from the group consisting of joint inflammation, arthritis, rheumatoid arthritis, rheumatoid spondylitis and osteoarthritis, sepsis, septic shock, endotoxic shock, Gram-negative sepsis, toxic shock syndrome, acute respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, asthma, bone resorption diseases, reperfusion injury, graft vs host reaction, allograft rejection, malaria, myalgias, HIV, AIDS, ARC, cachexia, Crohn's disease, ulcerative colitis, pyresis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes mellitus, psoriasis, Bechet's disease, anaphylactoid purpura nephritis, chronic glomerulonephritis, inflammatory bowel disease, and leukemia.

17. The method, according to claim 10, wherein the pathological condition or disease state is asthma.

18. The method, according to claim 16, wherein the disease state is selected from the group consisting of acute respiratory distress syndrome, pulmonary inflammatory disease, and pulmonary sarcoidosis.

19. The method, according to claim 16, wherein the disease state is selected from the group consisting of joint inflammation, arthritis, rheumatoid arthritis, rheumatoid spondylitis, and osteoarthritis.

20. The method, according to claim 14, wherein the disease state is a disease or disorder of the bran selected from the group consisting of brain trauma, ischaemia, Huntingdon's disease, and tardive dyskinesia.

21. The method, according to claim 14, wherein the disease state is a yeast or fungal infection.

22. A method for treating a human or animal in need of gastroprotection which comprises administering to said human or animal a gastroprotecting amount of a compound of claim 1 in association with a pharmaceutically-acceptable carrier.

23. A method for treating a human or animal in need of an analgesic, anti-tussive, or anti-hyperalgesic in the treatment of neurogenic inflammatory disease associated with irritation and pain which comprises administering to said human or animal an effective amount of a compound of claim 1 in association with a pharmaceutically-acceptable carrier.

* * * * *

Exhibit B

US005889014A

United States Patent [19]

Cavalla et al.

[11] **Patent Number: 5,889,014**

[45] **Date of Patent: Mar. 30, 1999**

[54] **HETEROCYCLIC COMPOUNDS FOR INHIBITING PHOSPHODIESTERASE IV**

[75] Inventors: **David John Cavalla**, Cambridge, England; **Lloyd J. Dolby**, Eugene, Oreg.; **Mark Chasin**, Manalapan, N.J.

[73] Assignee: **Euro-Celtique, S.A.**, Luxembourg, Luxembourg

[21] Appl. No.: **716,902**

[22] Filed: **Sep. 20, 1996**

Related U.S. Application Data

[60] Division of Ser. No. 370,952, Jan. 10, 1995, Pat. No. 5,591,776, which is a continuation-in-part of Ser. No. 321,730, Oct. 12, 1994, Pat. No. 5,665,737.

[51] **Int. Cl.**^{6} **A61K 31/505**; A61K 31/44; C07D 239/52; C07D 213/02

[52] **U.S. Cl.** **514/269**; 514/277; 514/354; 544/309; 544/311; 546/304; 546/309

[58] **Field of Search** 514/269, 277, 514/354; 544/309, 311; 546/304, 309, 311

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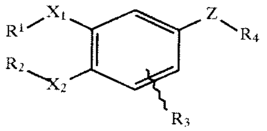
[57] **ABSTRACT**

Novel compounds which are effective PDE IV inhibitors are disclosed. The compounds have the formula:



wherein:

R_1 and R_2 may be the same or different and each is selected from the group consisting of hydrogen, saturated or unsaturated straight-chain or branched C_{1-12} alkyl groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety;

R_3 is hydrogen, halogen, or a saturated or unsaturated straight-chain or branched C_{1-12} alkyl group, a cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 7 carbon atoms in the cycloalkyl moiety;

R_4 is a phenyl or benzyl or a 6-membered heteroaryl which may be unsubstituted or substituted with one or more halogen atoms, alkyl groups, nitro groups, hydroxyl groups, cyano groups, carboxyl groups, alkoxy group, alkoxycarbonyl, amido, carboxamido, substituted or unsubstituted amino groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety, aryl or aralkyl groups preferably containing from about 6 to about 10 carbon atoms, or heterocyclic groups containing nitrogen, oxygen or sulfur in the ring; said alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, and arylalkyl groups being unsubstituted or substituted by halogen atoms, hydroxyl groups, cyano groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, carboxamido or substituted or unsubstituted amino groups, or one or more lower alkyl groups having from 1 to 3 carbon atoms;

Z is selected from the group consisting of $—CH_2CO—$, $—NHCH_2—$, $—CH_2NH—$, $—CH_2CONH—$, $—CH_2NHCO—$, $—CH_2COCH_2—$, $—COCH_2—$, $—C({=}NOC({=}O)NHQ)—$, and $—C({=}NQ)NH—$;

X_1 and X_2 may be the same or different and each is O or S;

wherein Q is R_4 or hydrogen except that when $Z{=}C({=}NOCONHQ)—$, R_4 is not benzyl and R_1 and R_2 are both not hydrogen.

16 Claims, No Drawings

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HETEROCYCLIC COMPOUNDS FOR INHIBITING PHOSPHODIESTERASE IV

This application is divisional of U.S. patent application Ser. No. 08/370,952, filed on Jan. 10, 1995, now U.S. Pat. No. 5,591,776, which is a continuation-in-part of U.S. patent application Ser. No. 08/321,730, filed Oct. 12, 1994, now U.S. Pat. No. 5,665,737.

BACKGROUND OF THE INVENTION

Asthma is a complex disease involving the concerted actions of multiple inflammatory and immune cells, spasmogens, inflammatory mediators, cytokines and growth factors. In recent practice there have been four major classes of compounds used in the treatment of asthma, namely bronchodilators (e.g., β-adrenoceptor agonists), anti-inflammatory agents (e.g., corticosteroids), prophylactic anti-allergic agents (e.g., cromolyn sodium) and xanthines (e.g., theophylline) which appear to possess both bronchodilating and anti-inflammatory activity.

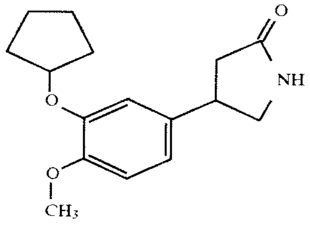
Theophylline has been a preferred drug of first choice in the treatment of asthma. Although it has been touted for its direct bronchodilatory action, theophylline's therapeutic value is now believed to also stem from anti-inflammatory activity. Its mechanism of action remains unclear. However, it is believed that several of its cellular activities are important in its activity as an anti-asthmatic, including cyclic nucleotide phosphodiesterase inhibition, adenosine receptor antagonism, stimulation of catecholamine release, and its ability to increase the number and activity of suppressor T-lymphocytes. While all of these actually may contribute to its activity, only PDE inhibition may account for both the anti-inflammatory and bronchodilatory components. However, theophylline is known to have a narrow therapeutic index, and a wide range of untoward side effects which are considered problematic.

Of the activities mentioned above, theophylline's activity in inhibiting cyclic nucleotide phosphodiesterase has received considerable attention recently. Cyclic nucleotide phosphodiesterases (PDEs) have received considerable attention as molecular targets for anti-asthmatic agents. Cyclic 3',5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP) are known second messengers that mediate the functional responses of cells to a multitude of hormones, neurotransmitters and autocoids. At least two therapeutically important effects could result from phosphodiesterase inhibition, and the consequent rise in intracellular adenosine 3',5'-monophosphate (cAMP) or guanosine 3',5'-monophosphate (cGMP)in key cells in the pathophysiology of asthma. These are smooth muscle relaxation (resulting in bronchodilation) and anti-inflammatory activity.

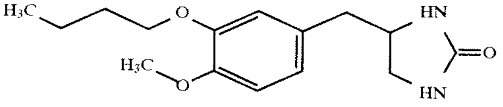
It has become known that there are multiple, distinct PDE iso-enzymes which differ in their cellular distribution. A variety of inhibitors possessing a marked degree of selectivity for one iso-enzyme or the other have been synthesized.

The structure-activity relationships (SAR) of isozyme-selective inhibitors has been discussed in detail, e.g., in the article of Theodore J. Torphy, et al., "Novel Phosphodiesterase Inhibitors For The Therapy Of Asthma", Drug News & Prospectives, 6(4) May 1993, pages 203–214. The PDE enzymes can be grouped into five families according to their specificity toward hydrolysis of cAMP or cGMP, their sensitivity to regulation by calcium, calmodulin or cGMP, and their selective inhibition by various compounds. PDE I is stimulated by Ca^{2+}/calmodulin. PDE II is cGMP-stimulated, and is found in the heart and adrenals. PDE III is cGMP-inhibited, and inhibition of this enzyme creates positive inotropic activity. PDE IV is cAMP specific, and its inhibition causes airway relaxation, anti-inflammatory and ant-depressant activity. PDE V appears to be important in regulating cGMP content in vascular smooth muscle, and therefore PDE V inhibitors may have cardiovascular activity.

While there are compounds derived from numerous structure activity relationship studies which provide PDE III inhibition, the number of structural classes of PDE IV inhibitors is relatively limited. Analogues of rolipram, which has the following structural formula:



and of RO-20-1724, which has the following structural formula:



have been studied.

Rolipram, which was initially studied because of its activity as an antidepressant has been shown to selectively inhibit the PDE IV enzyme and this compound has since become a standard agent in the classification of PDE enzyme subtypes. There appears to be considerable therapeutic potential for PDE IV inhibitors. Besides initial work suggesting an anti-depressive action, rolipram has been investigated for its anti-inflammatory effects, particularly in asthma. In-vitro, rolipram, RO20-1724 and other PDE IV inhibitors have been shown to inhibit (1) mediator synthesis/release in mast cells, basophils, monocytes and eosinophils; (2) respiratory burst, chemotaxis and degranulation in neutrophils and eosinophils; and (3) mitogen-dependent growth and differentiation in lymphocytes (The PDE IV Family Of Calcium-Phosphodiesterases Enzymes, John A. Lowe, III, et al., Drugs of the Future 1992, 17(9):799–807).

PDE IV is present in all the major inflammatory cells in asthma including eosinophils, neutrophils, T-lymphocytes, macrophages and endothelial cells. Its inhibition causes down-regulation of cellular activation and relaxes smooth muscle cells in the trachea and bronchus. On the other hand, inhibition of PDE III, which is present in myocardium, causes an increase in both the force and rate of cardiac contractility. These are undesirable side effects for an anti-inflammatory agent. Theophylline, a non-selective PDE inhibitor, inhibits both PDE III and PDE IV, resulting in both desirable anti-asthmatic effects and undesirable cardiovascular stimulation. With this well-known distinction between PDE isozymes, the opportunity for concomitant anti-inflammation and bronchodilation without many of the side effects associated with theophylline therapy is apparent. The increased incidence of morbidity and mortality due to asthma in many Western countries over the last decade has focused the clinical emphasis on the inflammatory nature of this disease and the benefit of inhaled steroids. Development of an agent that possesses both bronchodilatory and anti-inflammatory properties would be most advantageous.

It appears that selective PDE IV inhibitors should be more effective with fewer side effects than theophylline.

Attempts have therefore been made to find new compounds having more selective and improved PDE IV inhibition.

OBJECTS AND SUMMARY OF THE INVENTION

It is accordingly a primary object of the present invention to provide new compounds which are effective PDE IV inhibitors.

It is another object of the present invention to provide new compounds which act as effective PDE IV inhibitors with lower PDE III inhibition.

It is a further object of the present invention to provide new compounds which have a superior PDE IV inhibitory effect as compared to theophylline or other known compounds.

It is a further object of the present invention to provide new compounds which have a substantially equal or superior PDE IV inhibitory effect as compared to known chemical compounds, and which exhibit surprisingly greater selectivity with regard to their inhibitory effects.

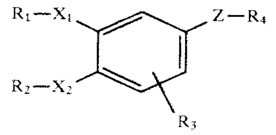
It is another object of the present invention to provide a method of treating a patient requiring PDE IV inhibition.

It is another object of the present invention to provide new compounds for treating disease states associated with abnormally high physiological levels of cytokines, including tumor necrosis factor.

It is another object of the present invention to provide a method of synthesizing the new compounds of this invention.

It is another object of the present invention to provide a method for treating a mammal suffering from a disease state selected from the group consisting of asthma, arthritis, allergies, inflammation, depression, dementia and disease states associated with abnormally high physiological levels of cytokines.

With the above and other objects in view, the present invention mainly comprises a compound of the formula:



wherein:

- X_1 and X may be the same or different and each is O or S;
- R_1 and R_2 may be the same or different and each is selected from the group consisting of hydrogen, saturated or unsaturated straight-chain or branched C_{1-12} alkyl groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety;
- R_3 is hydrogen, halogen, or a saturated or unsaturated straight-chain or branched C_{1-12} alkyl group, a cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 7 carbon atoms in the cycloalkyl moiety;
- Z is a linkage selected from $—NHCH_2—$, $—CH_2NH—$, $—CH_2CONH—$, $—CH_2NHCO—$, $—CH_2CO—$,

$—COCH_2—$, $—CH_2COCH_2—$, —C(=NQ)NH— and C(=NOCONHQ)—;
- R_4 is a phenyl or benzyl or a 6-membered heteroaryl group which may be unsubstituted or substituted with one or more halogen atoms, alkyl groups, hydroxyl groups, cyano groups, nitro groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, amido, carboxamido, substituted or unsubstituted amino groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety, aryl or aralkyl groups preferably containing from about 6 to about 10 carbon atoms, or heterocyclic groups containing nitrogen, oxygen or sulfur in the ring; said alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, and aryl-alkyl groups being unsubstituted or substituted by halogen atoms, hydroxyl groups, cyano groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, carboxamido or substituted or unsubstituted amino groups, or one or more lower alkyl groups having from 1 to 3 carbon atoms;
- Q is R_4 or hydrogen;
- except that when Z=—C(=NOCONHQ)—, R_4 is not benzyl; and R_1 and R_2 are both not hydrogen.

The term "lower alkyl" is defined for purposes of the present invention as straight or branched chain radicals having from 1 to 3 carbon atoms.

DETAILED DESCRIPTION

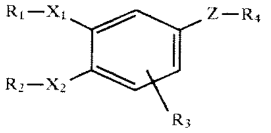
The compounds of the present invention, as demonstrated in the appended examples, are effective in the mediation or inhibition of PDE IV in humans and other mammals. Further, these compounds are selective PDE IV inhibitors which possess both bronchodilatory and anti-inflammatory properties substantially without undesirable cardiovascular stimulation caused by PDE III inhibition. Many of these compounds have a substantially equal or superior PDE IV inhibitory effect as compared to theophylline.

The present invention is further related to a method for the treatment of allergic and inflammatory disease which comprises administering to a mammal in need thereof an effective amount of the compounds of the present invention.

The present invention is also related to a method for the mediation or inhibition of the enzymatic or catalytic activity of PDE IV activity in mammals, particularly humans, which comprises administering an effective amount of the above-described compounds of the invention to a mammal in need of PDE IV inhibition.

The compounds of the present invention may find use in the treatment of other disease states in humans and other mammals, such as in the treatment of disease states associated with a physiologically detrimental excess of tumor necrosis factor (TNF). TNF activates monocytes, macrophages and T-lymphocytes. This activation has been implicated in the progression of Human Immuno-deficiency Virus (HIV) infection and other disease states related to the production of TNF and other cytokines modulated by TNF.

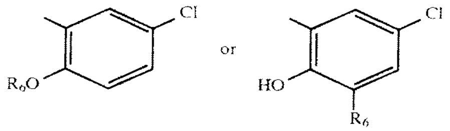
In certain preferred embodiments, the compounds of the present invention comprise the formula:



wherein:

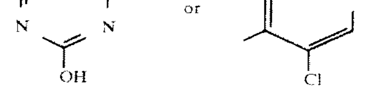
- X_1 and X_2 may be the same or different and each is O or S; and R_1 and R_2 may be the same or different and each is selected from the group consisting of hydrogen, saturated or unsaturated straight-chain or branched C_{1-12} alkyl groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety;
- R_3 is hydrogen, halogen, or a saturated or unsaturated straight-chain or branched C_{1-12} alkyl group, a cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 7 carbon atoms in the cycloalkyl moiety;
- Z is a linkage selected from —$NHCH_2$—, —CH_2NH—, —CH_2CONH—, —CH_2NHCO—, —CH_2CO—, —$COCH_2$—, —CH_2COCH_2—, —C(=NQ)NH— and C(=NOCONHQ)—;
- R_4 is a phenyl or benzyl or a 6-membered heteroaryl group which may be unsubstituted or substituted with one or more halogen atoms, alkyl groups, hydroxyl groups, cyano groups, nitro groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, amido, carboxamido, substituted or unsubstituted amino groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety, aryl or aralkyl groups preferably containing from about 6 to about 10 carbon atoms, or heterocyclic groups containing nitrogen, oxygen or sulfur in the ring; said alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, and aryl-alkyl groups being unsubstituted or substituted by halogen atoms, hydroxyl groups, cyano groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, carboxamido or substituted or unsubstituted amino groups, or one or more lower alkyl groups having from 1 to 3 carbon atoms;
- Q is R_4 or hydrogen;
- except that when Z=—C(=NOCONHQ)—, R_4 is not benzyl; and R_1 and R_2 are both not hydrogen.

In certain preferred embodiments, R_4 is a phenyl or substituted phenyl having one of the structures:



wherein R_6 is a substituted or unsubstituted lower alkyl having from about 1 to about 3 carbon atoms.

In another preferred embodiment R_4 is one of the following heteroaryl-groups having the structure:



In further preferred embodiments, Z is a linkage NHCO—, —$COCH_2$—, —CH_2CO—, —C(=NQ)NH— and —C(=NOCONHQ)—.

Certain preferred compounds of the present invention include:

(I) N-(3-Cyclopentyloxy-4-methoxyphenyl-acetyl)-2-allyloxy-5-chloroaniline;

(II) N-(3-Cyclopentyloxy-4-methoxyphenyl-acetyl)-2-hydroxy-3-allyl-5-chloroaniline;

(III) 1-(3-Cyclopentyloxy-4-methoxyphenyl)phenone-O-(aminocarbonyl)oxime;

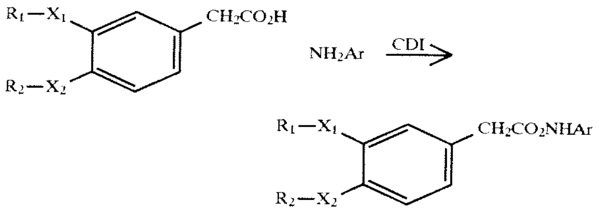
(IV) 1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-(pyrimid-2,4-dionyl))) ethanone; and

(V) N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

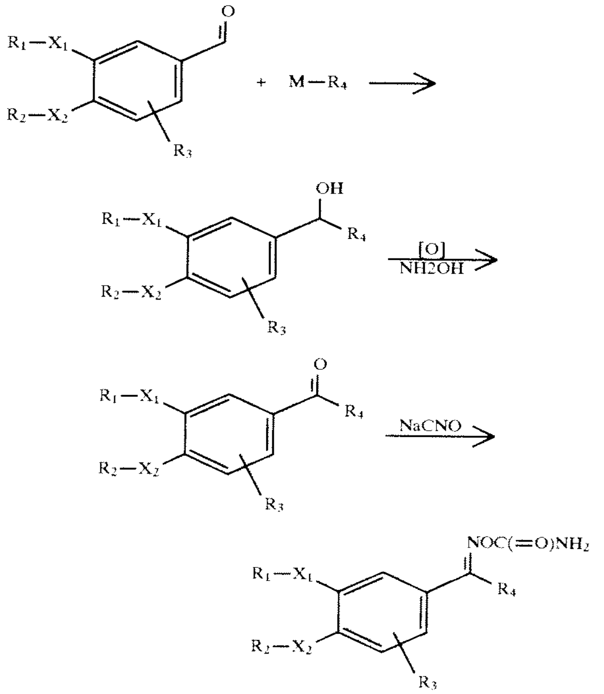
Representative processes for preparing the compounds of the present invention are shown below:

Detailed description of several syntheses are shown in the Examples.

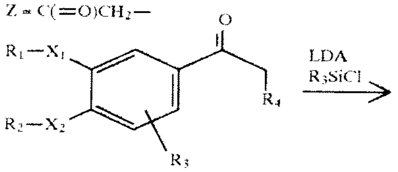
Z = CH_2CONH—



Z = C(=NOC(=O)NH_2)—



Z = C(=O)CH_2—



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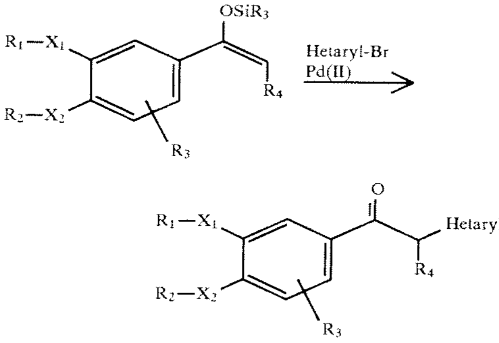
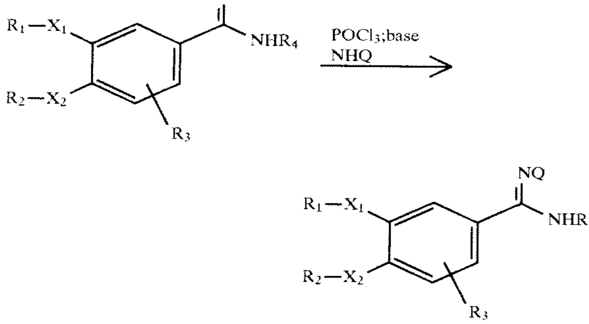


Z = C(=NQ)NH—



The compounds of the present invention have been found to be highly effective PDE IV inhibitors, the inhibition of which is in fact significantly and surprisingly greater than that of theophylline. The concentration which yields 50% inhibition of PDE IV (IC_{50}) for the compound prepared in Example 5 is 0.3 μM, whereas the IC_{50} for rolipram when run in the same assay was 2.8 μM. It is apparent that this inventive compound is several times as effective as a PDE IV inhibitor as compared to rolipram (or theophylline).

Since the PDE III IC_{50} of an Example 5 compound is >1000 μM, it is clear that the compound of the invention is highly selective as a PDE IV inhibitor.

Accordingly, the compounds of the present invention can be administered to anyone requiring PDE IV inhibition. Administration may be orally, topically, by suppository, inhalation or insufflation, or parenterally.

The present invention also encompasses all pharmaceutically acceptable salts of the foregoing compounds. One skilled in the art will recognize that acid addition salts of the presently claimed compounds may be prepared by reaction of the compounds with the appropriate acid via a variety of known methods. Alternatively, alkali and alkaline earth metal salts are prepared by reaction of the compounds of the invention with the appropriate base via a variety of known methods. For example, the sodium salt of the compounds of the invention can be prepared via reacting the compound with sodium hydride.

Various oral dosage forms can be used, including such solid forms as tablets, gelcaps, capsules, caplets, granules, lozenges and bulk powders and liquid forms such as emulsions, solution and suspensions. The compounds of the present invention can be administered alone or can be combined with various pharmaceutically acceptable carriers and excipients known to those skilled in the art, including but not limited to diluents, suspending agents, solubilizers, binders, disintegrants, preservatives, coloring agents, lubricants and the like.

When the compounds of the present invention are incorporated into oral tablets, such tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, multiply compressed or multiply layered. Liquid oral dosage forms include aqueous and nonaqueous solutions, emulsions, suspensions, and solutions and/or suspensions reconstituted from non-effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, coloring agents, and flavorings agents. When the compounds of the present invention are to be injected parenterally, they may be, e.g., in the form of an isotonic sterile solution. Alternatively, when the compounds of the present invention are to be inhaled, they may be formulated into a dry aerosol or may be formulated into an aqueous or partially aqueous solution.

In addition, when the compounds of the present invention are incorporated into oral dosage forms, it is contemplated that such dosage forms may provide an immediate release of the compound in the gastrointestinal tract, or alternatively may provide a controlled and/or sustained release through the gastrointestinal tract. A wide variety of controlled and/or sustained release formulations are well known to those skilled in the art, and are contemplated for use in connection with the formulations of the present invention. The controlled and/or sustained release may be provided by, e.g., a coating on the oral dosage form or by incorporating the compound(s) of the invention into a controlled and/or sustained release matrix.

Specific examples of pharmaceutically acceptable carriers and excipients that may be used for formulate oral dosage forms, are described in the *Handbook of Pharmaceutical Excipients,* American Pharmaceutical Association (1986), incorporated by reference herein. Techniques and compositions for making solid oral dosage forms are described in *Pharmaceutical Dosage Forms: Tablets* (Lieberman, Lachman and Schwartz, editors) 2nd edition, published by Marcel Dekker, Inc., incorporated by reference herein. Techniques and compositions for making tablets (compressed and molded), capsules (hard and soft gelatin) and pills are also described in *Remington's Pharmaceutical Sciences* (Arthur Osol, editor), 1553–1593 (1980), incorporated herein by reference. Techniques and composition for making liquid oral dosage forms are described in *Pharmaceutical Dosage Forms: Disperse Systems,* (Lieberman, Rieger and Banker, editors) published by Marcel Dekker, Inc., incorporated herein by reference.

When the compounds of the present invention are incorporated for parenteral administration by injection (e.g., continuous infusion or bolus injection), the formulation for parenteral administration may be in the form of suspensions, solutions, emulsions in oily or aqueous vehicles, and such formulations may further comprise pharmaceutically necessary additives such as stabilizing agents, suspending agents, dispersing agents, sustained release agents, and the like. The compounds of the invention may also be in the form of a powder for reconstitution as an injectable formulation.

The dose of the compounds of the present invention is dependent upon the affliction to be treated, the severity of the symptoms, the route of administration, the frequency of the dosage interval, the presence of any deleterious side-effects, and the particular compound utilized, among other things.

The PDE IV inhibitory compounds of the present invention may be examined for their PDE IV inhibitory effects via the techniques set forth in the following examples, wherein the ability of the compounds to inhibit PDE IV isolated from bovine tracheal smooth muscle is set forth.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples illustrate various aspects of the present invention, and are not to be construed to limit the claims in any manner whatsoever.

EXAMPLE 1

Preparation of N-(3-Cyclopentyloxy-4-methoxyphenyl-acetyl)-2-allyloxy-5-chloroaniline

a) 3-Cyclopentyloxy-4-methoxybenzyl Alcohol

A solution of 3-cyclopentyloxy-4-methoxybenzaldehyde (38 grams, 0.17 mol) in 40 milliliters of ethanol and sodium borohydride (1.63 grams,0.043 mol) was stirred for 2 hours at room temperature until complete by TLC. The reaction was diluted with water and extracted with ethyl acetate. Evaporation of the ethyl acetate afforded 3-cyclopentyloxy-4-methoxybenzyl alcohol (37 grams, 98%) suitable for the next step.

b) 3-Cyclopentyloxy-4-methoxybenzyl Chloride

A solution containing 3-cyclopentyloxy-4-methoxybenzyl alcohol (112 grams, 0.50 mol), prepared as described in step a), in 1 liter of methylene chloride was stirred at room temperature with concentrated HCl (110 milliliters, 1.2 mol) for 3 hours, at which time the reaction was done by TLC. The layers were separated and the methylene chloride solution was washed twice with water and evaporated under reduced pressure to give 3-cyclopentyloxy-4-methoxybenzyl chloride (119 grams, 100%).

c) 3-Cyclopentyloxy-4-methoxyphenylacetonitrile

A mixture of 3-cyclopentyloxy-4-methoxybenzyl chloride (119 grams, 0.49 mol), 120 milliliters of methylene chloride, KCN (70.7 grams, 1.09 mol), benzyltriethylammonium chloride (35 grams, 0.015 mol) and water (120 milliliters) was stirred vigorously at room temperature for 48 hours. The reaction mixture was diluted with. methylene chloride and the layers were separated. The methylene chloride solution was extracted several times with water and evaporated to yield 3-cyclopentyloxy-4-methoxyphenylacetonitrile (109 grams, 95%) for the subsequent transformation.

d) 3-Cyclopentyloxy-4-methoxyphenylacetic Acid

A solution of 3-cyclopentyloxy-4-methoxyphenylacetonitrile (109 grams, 0.43 mol) in 1330 milliliters of ethanol and NaOH (51 grams, 1.3 mol) was heated under reflux for 48 hours. Ethanol (500 milliliters) was distilled from the reaction mixture and the residue was diluted with water and stirred with Norit A (11 grams) for 2 minutes. The mixture was filtered through a pad of celite and acidified to pH 1 with concentrated HCl. Extraction of the mixture with diethyl ether yielded 120 grams of crude 3-cyclopentyloxy-4-methoxy-phenylacetic acid after evaporation of the ether at reduced pressure. The crude acid was dissolved in warm toluene (400 milliliters) and stirred for 1 hour with 10.5 grams of Norit A. The charcoal was filtered and the toluene solution was diluted with heptane (40 milliliters). Filtration of the cooled solution afforded 72 grams (67%) of pure 3-cyclopentyloxy-4-methoxyphenylacetic acid, mp 79–80°.

e) N-(3-Cyclopentyloxy-4-methoxy-phenylacetyl)-2-hydroxy-5-chloroaniline

A solution of 3-cyclopentyloxy-4-methoxyphenylacetic acid (10 grams, 0.040 mol) in 20 milliliters of methylene chloride was added dropwise to a stirred slurry of 1,1'-carbonyldiimidazole (7.1 grams, 0.044 mol) in 40 milliliters of methylene chloride. After stirring for 2 hours, the resulting solution was added to a solution of 2-hydroxy-5-chloraniline (6.0 grams, 0.042 mol) in methylene chloride (75 milliliters). After stirring overnight, water was added and stirring was continued. The layers were separated and the methylene chloride layer was washed with 100 milliliter portions of water, dilute aqueous HCl, and water. Evaporation of the methylene chloride afforded the solid amide which was triturated with methanol (20 milliliter) and filtered to give 10.7 grams (71%) of N-(3-cyclopentyloxy-4-methoxyphenyl-acetyl)-2-hydroxy-5-chloraniline, mp 151–152°.

f) N-(3-Cyclopentyloxy-4-methoxyphenyl-acetyl)-2-allyloxy-5-chloroaniline

In this step, allyl chloride (23.3 grams, 0.31 mol) was added to a stirred solution of N-(3-cyclopentyloxy-4-methoxy-phenylacetyl)-2-hydroxy-5-chloroaniline (78 grams, 0.21 mol) in ethanol (600 millilters) and 1N NaOH in methanol (213 milliters). The mixture was heated under reflux for 8 hours and then diluted with water and extracted twice with ethyl acetate. Evaporation of the ethyl acetate and crystallization of the residue from methanol gave 56.4 grams (65%) of the title compound, mp 75–76.5°.

EXAMPLE 2

Preparation of N-(3-Cyclopentyloxy-4-methoxyphenyl-acetyl)-2-hydroxy-3-allyl-5-chloroaniline

A solution of N-(3-cyclopentyloxy4-methoxyphenyl-acetyl)-2-allyloxy-5-chloroaniline (33 grams, 0.079 mol) in 330 millimeters of diphenyl ether was heated at 180° for 6.5 hours. This reaction mixture was combined with a second reaction mixture containing N-3-cyclopentyloxy-4-methoxyphenylacetyl)-2-allyloxy-5-chloroaniline and 250 milliliters of diphenyl ether which had been heated at 180° for 20 hours.

The combined reaction mixtures were diluted with 800 milliliters of hexane and applied to a flash chromatography column prepared from 470 grams of flash chromatography silica gel. Fractions of 800 milliliters were collected. Fractions 1–6 (hexane) contained only diphenyl ether. Fraction 7 (1:1 hexane/methylene chloride) contained 1 gram of material. Fractions 8–11 (methylene chloride) contained 34.5 grams of material with a higher R_f on TLC (2% methanol/methylene chloride, silica gel) than the title compound Fractions 12–17 (methylene chloride) gave 20 grams (34%) of the crude title compound. A sample of this material (1.0 grams) was recrystallized from 10 milliliters of ethanol to give the pure title compound, mp 119°–120°.

EXAMPLE 3

Preparation of 1-(3-Cyclopentyloxy-4-methoxyphenyl)phenone-O-(aminocarbonyl)oxime

a) α-Phenyl-3-cyclopentyloxy-4-methoxybenzyl alcohol

Phenyllithium (1.8M solution in cyclohexane/diethyl ether, 25.5 milliliters, 46 mmol) was added dropwise over 15 minutes to a stirred solution of 3-cyclopentyloxy-4-methoxybenz-aldehyde (6 grams, 27 mmol) in dry tetrahydrofuran (20 milliliters) at −78° C. The resulting solution was stirred at −78° C. for 30 minutes and quenched at −78° C. by the rapid addition of aqueous saturated NH_4Cl (70 milliliters). After warming to room temperature, water was added to dissolve the solids and volatiles were removed in vacuo. The residue was partitioned between water (250 milliliters) and ethyl acetate (250 milliliters), the aqueous phase was extracted with ethyl acetate (3×250 milliliters) and the combined organic layers washed with water (200 milliliters). The organics were dried over Na_2SO_4 and concentrated in vacuo to give a light yellow oil. The oil was purified by flash chromatography (SiO_2:hexane/ethyl acetate (4:1)) to afford the title compound as a pale yellow oil (7.4 grams).

^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.30 (m, 5H), 6.84 (m,3H), 5.76 (s, 1H), 4.72 (m, 1H), 3.81 (s, 3H), 2.26 (s, 1H), 1.85 (m, 6H), 1.59 (m, 2H).

b) 1-(3-Cyclopentyloxy-4-methoxyphenyl)phenone

Pyridinium dichromate (13.22 grams, 35.19 mmol) was added in one portion to a stirred solution of α-phenyl-3-cyclopentyloxy-4 -methoxybenzyl alcohol (7.0 grams, 23.5 mmol) in dry methylene chloride (200 milliliters) at room temperature. The resulting heterogeneous solution was stirred at room temperature overnight. The reaction mixture was diluted with an equal volume of diethyl ether and stirred for 1 hour. The mixture was filtered through celite and the filter cake washed with diethyl ether (150 milliliters) and ethyl acetate (150 milliliters). The brown filtrate was concentrated in vacuo and purified by flash chromatography (SiO_2:hexane/ethyl acetate (7:3) to yield the title compound as an orange solid (6.940 grams). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.75 (m, 2H), 7.46 (m,5H), 5.76 (s, 1H), 4.72 (m, 1H), 3.81 (s, 3H), 2.26 (s, 1H), 1.85 (m, 6H), 1.59 (m, 2H).

c) 1-(3-Cyclopentyloxy-4-methoxyphenyl)phenone Oxime

Hydroxylamine hydrochloride (1.179 grams, 25.7 mmol) was added in one portion to a magnetically stirred solution of 1-(3-cyclopentyloxy-4-methoxyphenyl)phenone (6.936 grams, 23.4 mmol) in dry pyridine (120 milliliters) at room temperature. The resulting suspension slowly became homogeneous and the solution was stirred at room temperature overnight. Further hydroxylamine hydrochloride (0.5 grams, 7.17 mmol) was added to the reaction mixture and stirring continued overnight. The pyridine was removed in vacuo and the residue partitioned between ethyl acetate (200 milliliters) and water (200 milliliters). The aqueous phase was extracted with ethyl acetate (2×125 milliliters), the organics dried over Na_2SO_4 and concentrated in vacuo to give an orange oil. The oil was purified by flash chromatography (SiO_2:hexane/ethyl acetate (4:1)) to afford the title compound as a tan solid (2.49 grams).

R_f(SiO_2:ethyl acetate/hexane (3:7)) 0.25

d) 1-(3-Cyclopentyloxy-4-methoxy-phenyl)phenone-O-(aminocarbonyl)oxime

Anhydrous trifluoroacetic acid (2.45 milliliters, 3.64 grams, 31.92 mmol) was addded dropwise over 10 minutes at room temperature to a slowly stirred suspension of sodium cyanate (4.15 grams, 63.84 mmol) in methylene chloride (30 milliliters). The suspension slowly thickened to a gelatinous mass which was periodically agitated by hand. After 30 minutes at room temperature, 1-(3-cyclopentyloxy-4-methoxyphenyl)phenone oxime (2.49 grams, 7.99 mmol) in methylene chloride (10 milliliters) was added in one portion and the reaction mixture stirred under nitrogen for 1 hour. The reaction mixture was poured into saturated $NaHCO_3$ (100 milliliters) and extracted with methylene

chloride (2×200 milliliters). The organic phase was washed with water (100 milliliters), dried (Na_2SO_4) and concentrated in vacuo to give a pale yellow oil. The oil was purified by flash chromatography (SiO_2:methylene chloride/ethyl acetate (9:1)) to yield the title compound as a white solid (1.21 grams). m.p. 129–132° C.

^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.43 (m, 5H), 6.94 (m,3H), 4.69 (m, 1H), 3.86 (3, 3H), 1.78 (m, 6H), 1.60 (m, 2H).

EXAMPLE 4

Preparation of 1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-(pyrimid-2,4-dionyl))) ethanone

a) [[1-(3-Cyclopentyloxy-4-methoxyphenyl)-1-ethenyl]oxy]trimethylsilane

Lithium diisopropylamide (1.5M solution in cyclohexane, 12.5 milliliters, 18.75 mmol) was added over 3 minutes to a stirred solution of 3-cyclopentyloxy-4-methoxyacetophenone (3 grams, 12.8 mmol) in dry tetrahydrofuran (10 milliliters) at −78° C. After the addition was complete, the solution was stirred for 30 minutes at −78° C. and trimethylsilyl chloride (2.4 milliliters, 30.72 mmol) was added in one portion. The mixture was allowed to warm to room temperature, stirred for 20 minutes, and quenched with saturated $NaHCO_3$ (35 milliliters). The resulting mixture was extracted with hexane (2×50 milliliters), the organic layer dried (Na_2SO_4) and the solvent removed in vacuo to afford the title compound as a colorless oil (4 grams); 80% pure by gas chromatography/mass spectrometry.

b) 1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-(pyrimid-2,4-dionyl)))ethanone

A stirred solution of ([1-(3-cyclopentyloxy-4-methoxyphenyl)-1-ethenyl)oxy]trimethylsilane (4.0 grams, 80% pure by gas chromatography, 10.3 mmol), 5-bromouracil (1.73 grams, 9.14 mmol), tributyltin fluoride (2.8 grams, 9.14 mmol) and bis(triphenylphosphine) palladium (II) chloride (215 milligrams, 3.0×10^{-4} mmol) in 1,4 dioxane (50 milliliters) was refluxed for 60 hours. The reaction mixture was cooled to room temperature, diluted with diethyl ether (150 milliliters) and washed with 1N NaOH (3×60 milliliters). The aqueous layer was neutralized with 12N HCl and extracted with methylene chloride (3×50 milliliters). The organics were dried (Na_2SO_4) and the solvent removed in vacuo to afford an oil. The oil was purified by chromatography on SiO_2 eluting with methylene chloride/ethanol/ammonia (9:1:0.1) to yield the title compound as a white solid (70 milligrams).

^{1}H NMR ($CDCl_3$; 250 MHz) δ 9.28 (brs, 1H), 8.87 (brs, 1H), 7.16 (s, 1H), 6.84 (m, 3H), 5.69 (s, 1H), 5.46 (s, 1H), 4.75 (m, 1H), 3.83 (s, 3H), 1.81 (m, 6H), 1.57 (m, 2H).

EXAMPLE 5

N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine

A solution of N-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamide (1 gram, 2.62 mmol) in phosphoryl chloride (15 milliliters, 161 mmol) and N, N-dimethylaniline (0.75 milliliters) was refluxed under nitrogen for 20 hours. The mixture was cooled to room temperature, volatiles removed in-vacuo, and the brown residue azeotroped with toluene (3×20 milliliters). The crude imino chloride was dissolved in toluene (15 milliliters), an excess of benzylamine (2.57 milliliters, 23.58 mmol) was added in one portion and the mixture refluxed, under nitrogen, for 4 hours. The mixture was cooled to room temperature, volatiles removed in-vacuo, and the residue dissolved in chloroform (100 milliliters) and washed with water (2×50 milliliters). The combined organics were dried (Na_2SO_4) and volatiles removed in-vacuo to yield a white solid. The mixture was purified by flash chromatography (SiO_2; ethyl acetate : hexane (1:9), followed by ethyl acetate : hexane (1:4)) to yield the title compound (624 milligrams, 50%) as a white crystalline solid, m.p. 129–133° C.

δ_H (250 MHz; $CDCl_3$) 1.63 (8H, m, 4 x CH_2), 3.78 (3H, s, OMe), 4.46 (1H, m, CH), 4.74 (2H, d, $ArCH_2$), 5.38 (1H, t, NH), 6.71 (2H, m, 2 x ArH), 6.95 (1H, dd, ArH), 7.38 (5H, m, 5 x ArH), 8.20 (2H, s, 2 x ArH).

EXAMPLE 6

Protocols for PDE III and PDE IV inhibition activity are set forth below:

Type III Phosphodiesterase

Enzyme Isolation Protocol

The Type III PDE is isolated from human platelets using a procedure similar to that previously described by Weishaar, R. E., et al., Biochem. Pharmacol., 35:787, 1986. Briefly, 1–2 units of platelets are suspended in an equal volume of buffer (20 mM Tris-HCl, pH 7.5, containing 2 mM magnesium acetate, 1 mM dithiothreitol, and 5 mM Na_2 EDTA). The proteinase inhibitor phenylmethyl-sulfonyl fluoride (PMSF) is also included in this buffer at a final concentration of 200 μM. The suspension is homogenized using a polytron and the homogenate centrifuged at 100,000 x g for 60 minutes. This and all subsequent procedures are performed at 0–4° C. The supernatant is then filtered through four layers of gauze and applied to a DEAE-Trisacryl M column, previously equilibrated with buffer B (20 mM Tris-HCl, pH 7.5, containing 1 mM magnesium acetate, 1 mM dithiothreitol and 200 μM PMSF). After application of the sample, the column is washed with several bed volumes of buffer B, after which the different forms of PDE are eluted from the column using two successive linear NaCl gradients (0.05–0.15M, 300 milliliters total; 0.15–0.40M, 200 milliliters total). Five milliliter fractions are collected and assayed for cyclic AMP and cyclic GMP PDE activity. Fractions containing PDE III activity are pooled and dialyzed overnight against 4 liters of buffer B. The dialyzed PDE III is then concentrated to 10% of the original volume, diluted to 50% with ethylene glycol monoethyl ether and stored at −20° C. PDE III can typically be retained for up to four weeks with little or no loss of activity.

Measuring Type III PDE Activity

Enzyme activity is assessed by measuring the hydrolysis of [^{3}H]-cyclic AMP, as described by Thompson, W. J., et al., Adv. Cyclic Nucleotide Res. 10:69, 1979. The cyclic AMP concentration used in this assay is 0.2 μM, which approximates to the K_m value. Protein concentration is adjusted to ensure that no more than 15% of the available substrate is hydrolyzed during the incubation period.

All test compounds are dissolved in dimethyl sulfoxide (final concentration of 2.5%). This concentration of dimethyl sulfoxide inhibits enzyme activity by approximately 10%.

Type IV Phosphodiesterase

Enzyme Isolation Protocol

The Type IV PDE is isolated from bovine tracheal smooth muscle using a procedure similar to that previously described by Silver, P. J., et al. Eur. J. Pharmacol. 150:85, 1988. Briefly, smooth muscle from bovine trachea is minced and homogenized using a polytron in 10 volumes of an extraction buffer containing 10 mM Tris-acetate (pH 7.5), 2 mM magnesium chloride, 1 mM dithiothreitol and 2,000 units/milliliters of aprotinin. This and all subsequent procedures are performed at 0–4° C. The homogenate is sonicated and then centrifuged at 48,000 x g for 30 minutes. The resulting supernatant is applied to a DEAE Trisacryl M column previously equilibrated with sodium acetate and dithiothreitol. After applications of the sample, the column is washed with sodium acetate/dithiothreitol, after which the different forms of PDE are eluted from the column using a linear Tris-HCl/NaCl gradient. Fractions containing Type IV PDE are collected, dialyzed and concentrated to 14% of the original volume. The concentrated fractions are diluted to 50% with ethylene glycol and stored at −20° C.

Measuring Type IV PDE Activity

Enzyme activity is assessed by measuring the hydrolysis of [^{3}H]-cyclic AMP, as described by Thompson, W. J., et al., Adv. Cyclic Nucleotide Res. 10:69, 1979. The cyclic AMP concentration used in this assay is 0.2 μM, which approximates the K_m value. Protein concentration is adjusted to ensure that no more than 15% of the available substrate is hydrolyzed during the incubation period.

All test compounds are dissolved in dimethyl sulfoxide (final concentration of 2.5%). This concentration of dimethyl sulfoxide inhibits enzyme activity by approximately 10%.

EXAMPLE 7

Following the above procedures, the PDE III and PDE IV inhibition for the compounds of Examples 1–5, and rolipram were tested and compared. The results are shown in the Table below:

TABLE

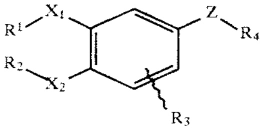
	IC_{50} (μM)	
Compound	PDE III ACTIVITY	PDE IV ACTIVITY
Example 1	>1000	2.2
Example 2	>1000	2.8
Example 3	89.5	1.8
Example 4	205.4	3.2
Example 5	>1000	0.3
Rolipram	790	2.8

Thus, it can be seen from the foregoing that compounds prepared in accordance with the present invention have high levels of PDE IV inhibition while, at the same time relatively low levels of PDE III inhibition.

While the invention has been illustrated with respect to the production and use of a particular compound, it is apparent that variations and modifications of the invention can be made without departing from the spirit or scope of the invention.

What is claimed is:

1. A compound of the formula:



wherein:

R_1 is C_{1-12} alkyl or cycloalkyl of 3–6 carbon atoms, said cycloalkyl may be substituted by one or more alkyl groups or by one or more halogens;

R_2 is C_{1-12} alkyl;

R_3 is hydrogen, lower alkyl or halogen;

R_4 is a 6-membered heteroaryl which may be unsubstituted or substituted with one or more halogen atoms, alkyl groups, nitro groups, hydroxyl groups, cyano groups, carboxyl groups, alkoxy group, alkoxycarbonyl, amido, carboxamido, substituted or unsubstituted amino groups, cycloalkyl and cycloalkyl-alkyl groups containing from 3 to 10 carbon atoms in the cycloalkyl moiety, aryl or aralkyl groups, or heterocyclic groups containing nitrogen, oxygen or sulfur in the ring; said alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, and arylalkyl groups being unsubstituted or substituted by halogen atoms, hydroxyl groups, cyano groups, carboxyl groups, alkoxy groups, alkoxycarbonyl, carboxamido or substituted or unsubstituted amino groups, or one or more lower alkyl groups having from 1 to 3 carbon atoms;

Z is selected from the group consisting of —C(=NOC(=O)NHQ)—, and —C(=NQ)NH—;

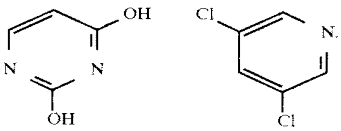
X_1 and X_2 may be the same or different and each is O or S;

wherein Q is R_4, benzyl or hydrogen.

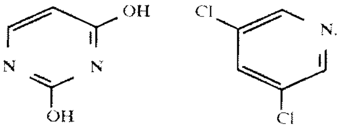
2. A compound of claim 1, wherein Z is C(=NOC(=O)NHQ)—.

3. A compound of claim 1, wherein Z is —C(=NQ)NH—.

4. A compound of claim 2, wherein R_4 is one of:



5. A compound of claim 3, wherein R_4 is one of:



6. A pharmaceutical composition comprising the compound of claim 1.

7. A pharmaceutical composition of claim 5, wherein said compound is selected from the group consisting of:

1-(3-cyclopentyloxy-4-methoxphenyl)-(2-(5-(pyrimid-2,4-dionyl)ethanone; and

N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

8. A method of treating a mammal suffering from a disease state selected from the group consisting of asthma, allergies, inflammation, depression, dementia, atopic diseases, rhinitis, and disease states associated with abnormally high physiological levels of cytokine, comprising administering an effective amount of the compound of claim 1.

9. The method of claim 8, wherein said compound is selected from the group consisting of 1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-pyrimid-2,4-dionyl)) ethanone; and N-Benzyl-N'(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

10. A method of effecting selective PDE IV inhibition to a patient requiring the same, comprising administering an effective amount of the compound of claim 1.

11. The method of claim 9, wherein said compound is selected from the group consisting of:

1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-(pyrimid-2,4-dionyl))) ethanone; and

N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

12. The method of claim 7, wherein said compound is selected from the group consisting of:

1-(3-cyclopentyloxy-4-methoxyphenyl)-(2-(5-(pyrimid-2,4-dionyl))) ethanone; and

N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

13. N-Benzyl-N'-(3,5-dichloropyrid-4-yl)-3-cyclopentyloxy-4-methoxybenzamidine.

14. A pharmaceutical composition comprising the compound of claim 13.

15. A method of treating a mammal suffering from a disease state selected from the group consisting of asthma, allergies, inflammation, depression, dementia, atopic diseases, rhinitis, and disease states associated with abnormally high physiological levels of cytokine, comprising administering an effective amount of the compound of claim 13.

16. A method of effecting selective PDE IV inhibition to a patient requiring the same, comprising administering an effective amount of the compound of claim 13.

* * * * *

Exhibit C

US005998428A

United States Patent [19]

Barnette et al.

[11] **Patent Number: 5,998,428**

[45] **Date of Patent: Dec. 7, 1999**

[54] **COMPOUNDS AND METHODS FOR TREATING PDE IV-RELATED DISEASES**

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[21] Appl. No.: **08/944,044**

[22] Filed: **Sep. 3, 1997**

Related U.S. Application Data

[63] Continuation of application No. 08/456,274, May 31, 1995, which is a continuation of application No. PCT/US94/06861, Jun. 17, 1994.

[51] **Int. Cl.^{6}** **A61K 31/44**
[52] **U.S. Cl.** **514/285**
[58] **Field of Search** 514/285

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Primary Examiner—Keith D. MacMillan
Attorney, Agent, or Firm—James M Kanagy; Charles M Kinzig

[57] **ABSTRACT**

This invention relates to a method for selecting PDE IV inhibitors which have a salutory therapeutic index, and to compounds having these properties.

17 Claims, No Drawings

COMPOUNDS AND METHODS FOR TREATING PDE IV-RELATED DISEASES

This is a continuation of application Ser. No. 08/456,274, filed May 31, 1995, which is a continuation of PCT/US94/06861, filed Jun. 17, 1994.

SCOPE OF THE INVENTION

This invention covers compounds which preferentially inhibit, or bind, one form of a phosphodiesterase isozyme denominated IV (PDE IV hereafter) while exhibiting equal or, preferably less binding or inhibition for a second form of the enzyme. These forms, and it is believed they are different forms of non-interconvertible conformations of the same enzyme though this has not been proven, are distinguished by their binding affinity for rolipram, an architypical PDE IV inhibitor. Rolipram binds with high affinity to the catalytic site of one form but with low affinity to the catalytic site of the other. Herein one form is denominated the high affinity rolipram binding site and the other form is identified as the low affinity rolipram binding site. A method for selectively treating diseases related to inhibiting preferentially the low affinity form of the catalytic site in the PDE IV isozyme is also disclosed. A method for treating certain diseases comprising administering a compound preferentially binding to the low affinity binding site is also disclosed.

AREA OF THE INVENTION

Cyclic nucleotide phosphodiesterases (PDEs) represent a family of enzymes that hydrolyze the ubiquitous intracellular second messengers, adenosine 3',5'-monophosphate (cAMP) and guanosine 3',5'-monophosphate (cGMP) to their corresponding inactive 5'-monophosphate metabolites. At least five distinct classes of PDE isozymes are believed to exist, each possessing unique physical and kinetic characteristics and each representing a product of a different gene family. These have been distinguished using the Roman numerals I through V.

The target enzyme in this invention is the PDE IV isozyme in all its various forms and in the full domain of its distributions in all cells. It is a low K_m (cAMP K_m=1–5 μM) cAMP-selective enzyme that has little activity against cGMP (Km>100 μM). Members of this isozyme class have the interesting characteristics of existing in two or more non-interconvertible or slowly interconvertible forms that bind rolipram and other PDE IV inhibitors with distinct rank order potencies. Thus the same gene product can exist in more than one catalytically active conformational state. Importantly, the relative proportions of the different binding forms may vary depending on the tissue cell type. For example, inflammatory cells may contain a relatively high proportion of the form that binds rolipram with a low affinity while brain and parietal cells may contain a relatively high proportion of the form that binds rolipram with a high affinity.

Of particular interest in this invention is the role this class of isozymes play in inflammation and airway smooth muscle. Studies indicate that it plays a prominent role in regulating cAMP in a wide variety of inflammatory cells (i.e., mast cells, basophils, eosinophils, neutrophils, and monocytes) and airway smooth muscle. The work of this invention is particularly applicable to inflammatory cells and airway smooth muscle; the isozyme type expressed in human monocytes is of particular interest. This is because cyclic AMP serves as a second messenger to inhibit chemotaxis and activation of inflammatory cells. In addition, cAMP mediates smooth airway muscle relaxation. This coupled with the major role of PDE IV in metabolizing cAMP has provided the underpinnings for investigating PDE IV inhibitors: by virtue of their ability to elevate cAMP content in leukocytes and airway smooth muscle, PDE IV inhibitors may posses anti-inflammatory and bronchodilator activities.

Current PDE inhibitors used in treating inflammation and as bronchodilators, drugs like theophylline and pentoxyfyllin, inhibit PDE isozymes indiscriminently in all tissues. These compounds exhibit side effects, apparently because they non-selectively inhibit all 5 PDE isozyme classes in all tissues. This is a consideration in assessing the therapeutic profile of these compounds. The targeted disease state may be effectively treated by such compounds, but unwanted secondary effects may be exhibited which, if they could be avoided or minimized, would increase the overall therapeutic effect of this approach to treating certain disease states. Taken collectively, this information suggests that the side effects associated with the use of standard non-selective PDE inhibitors might be reduced by targeting novel isozyme-selective inhibitors for the predominant PDE in the tissue or cell of interest. Although in theory isozyme-selective PDE inhibitors should represent an improvement over non-selective inhibitors, the selective inhibitors tested to date are not devoid of side effects produced as an extension of inhibiting the isozyme of interest in an inappropriate or not-targeted tissue. For example, clinical studies with the selective PDE IV inhibitor rolipram, which was being developed as an antidepressant, indicate it has psychotropic activity and produces gastrointestinal effects, e.g., pyrosis, nausea and emesis. Indications are that side effects of denbufylline, another PDE IV inhibitor targeted for the treatment of multi-infarct dementia, may include pyrosis, nausea and emesis as well. These side effects are thought to occur as a result of inhibiting PDE IV in specific areas of the CNS and gastrointestinal system.

In 1986, Schneider and colleagues described the presence and characteristics of high affinity, stereoselective [^{3}H]-rolipram binding sites in rat brain homogenates. Although it was assumed that these binding sites represented the catalytic site of the rat brain "non-calmodulin-dependent, cAMP phosphodiesterase" (i.e. PDE IV), a striking anomaly was apparent in the data. Under similar albeit not identical experimental conditions, data showed rolipram had a K_d=1 nM, whereas it inhibited rat brain PDE IV activity with a K_i=1 μM. Thus, there was a 1000-fold difference in the affinity of rolipram for the binding site versus its affect on catalytic activity. Although comprehensive structure activity relationships (SARs) for PDE inhibition and competition for [^{3}H]-rolipram binding were not established, the substantial difference in the potency of rolipram as a PDE IV inhibitor compared with its potency at the binding site seemed to question the validity of the assumption that both activities were contained within the same molecular locus.

Because of this conundrum, several studies were initiated. One sought to determine whether rolipram's high affinity binding site existed on the same protein as the cAMP catalytic site. Another study sought to determine whether or not the SAR for inhibition of PDE IV was the same as the SAR for competition with the high affinity rolipram binding site. A third study undertook to try and elucidate what biological significance, if any, there might be in these findings, particularly as it might relate to developing new drug therapies.

As data were collected from several assays, it became apparent that there are at least two binding forms on human monocyte recombinant PDE IV (hPDE IV) at which inhibitors bind. One explanation for these observations is that hPDE IV exists in two distinct forms. One binds the likes of rolipram and denbufylline with a high affinity while the other binds these compounds with a low affinity. Herein we distinguish these forms by referring to them as the high affinity rolipram binding form (HPDE IV) and the low affinity rolipram binding form (LPDE IV).

The importance of this finding lies in the discovery that compounds which potently compete for the high affinity rolipram binding form (HPDE IV) have more side effects or more intense side effects than those which more potently compete with the LPDE IV (low affinity rolipram binding form). Further data indicate that compounds can be targeted to the low affinity binding form of PDE IV and that this form is distinct from the binding form for which rolipram is a high affinity binder. Distinct SARs were found to exist for inhibitors acting at the high affinity rolipram binding form versus the low affinity rolipram binding form. In addition, these two forms appear to have different functional roles. Thus compounds that interacted with the low affinity rolipram binding form appear to have anti-inflammatory activity, whereas those that interact with the high affinity rolipram binding form produce side effects or exhibit more intensely those side effects.

There is no clear explanation for these findings. However, it is proposed the PDE IV can exist in two distinct tertiary or quaternary states. Both forms are believed to be catalytically active. Rolipram binds to one catalytic site of one form with a high affinity, defined herein as having a K_i less than 10 nanomolar, and to the other form with a low affinity, defined here as having a K_i of greater than 100 nanomolar.

A useful consequence of these findings is that it is now possible to identify compounds which preferentially inhibit cAMP catalytic activity where the enzyme is in the form that binds rolipram with a low affinity, thereby reducing the side effects which apparently are linked to inhibiting the form which binds rolipram with a high affinity. This provides a superior therapeutic index vis-a-vis anti-inflammatory and/or bronchodilator activities versus side effects.

SUMMARY OF THE INVENTION

In a first aspect, this invention relates to a compound which has an IC_{50} ratio of about 0.1 or greater as regards the IC_{50} for the PDE IV catalytic form which binds rolipram with a high affinity divided by the IC_{50} for the form which binds rolipram with a low affinity.

Furthermore, this invention relates to a method for elevating cAMP in leukocytes and airway smooth muscles while minimizing gastrointestinal and psychotropic effects, which method comprises administering to a subject in need thereof an effective amount of a compound having an IC_{50} ratio of about 0.1 or greater as regards the IC_{50} for PDE IV catalytic form which binds rolipram with a high affinity divided by the IC_{50} for the form which binds rolipram with a low affinity.

In yet another aspect, this invention relates to a method for treating inflammation or for dilating bronchi by preferentially inhibiting the PDE IV catalytic form that binds rolipram with a low affinity, which method comprises administering to a subject in need thereof a therapeutically effective amount of a compound which has an IC_{50} ratio of about 0.1 or greater as regards the IC_{50} for PDE IV catalytic form which binds rolipram with a high affinity divided by the IC_{50} for the form which binds rolipram with a low affinity.

DETAILED DESCRIPTION OF THE INVENTION

For purposes of this invention, the cAMP catalytic site which binds rolipram with a low affinity is denominated the "low affinity" binding site (LPDE IV) and the other form of this catalytic site which binds rolipram with a high affinity is denominated the "high affinity" binding site (HPDE IV).

Initial experiments were conducted to establish and validate a [3H]-rolipram binding assay. Details of this work are given in Example 1 below.

To determine whether both the high affinity binding activity and the low affinity binding activity resided in the same gene product, yeast were transformed by known methods and the expression of recombinant PDE IV was followed over a 6 hour fermentation period. Western blot analysis using an antibody directed against PDE TV indicated that the amount of PDE IV expressed increased with time, reaching a maximum after 3 hour of growth. In addition, greater than 90% of the immunoreactive product was in the high speed (100,000×g) supernatant of yeast lysates. [^{3}H] R-(−)-Rolipram binding and PDE activity were monitored along with protein expression. PDE IV activity was co-expressed with rolipram binding activity, indicating that both functions exist on the same gene product. Similar to results with the Western plot analysis, greater than 85% of the rolipram-inhibitable PDE activity and [^{3}H]-rolipram binding activity was found to be present in the yeast supernatant fraction.

Overall, most of the recombinant PDE IV expressed in this system exists as LPDE IV and only a small fraction as HPDE IV. Consequently, inhibition of recombinant PDE IV catalytic activity primarily reflects the actions of compounds at LPDE IV. Inhibition of PDE IV catalytic activity can thus be used as an index of the potency of compounds at LPDE IV. The potency of compounds at HPDE IV can be assessed by examining their ability to compete for [^{3}H]R-rolipram. To develop SARs for both the low affinity and high affinity rolipram binding sites, the potencies of selected compounds were determined in two assay systems. Results from experiments using standard compounds were tabulated. As expected, certain compounds were clearly more potent in competing with [^{3}H]-rolipram at the site for which rolipram demonstrated high affinity binding as compared with the other site, the one at which rolipram is a low affinity binder. SAR correlation between high affinity binding and low affinity binding was poor and it was concluded that the SAR for inhibition of high affinity [^{3}H]-rolipram binding was distinct from the SAR for binding to the low affinity rolipram binding site. Table I provides results from this SAR work.

TABLE I

Compound	Low Affinity IC_{50}	High Affinity IC_{50}	High/Low Ratio
1-(5-tetrazole)-3-(3-cyclopentyloxy-4-methoxyphenyl)cyclopentane	1.1	0.002	0.0018
cis-[3-(3-cyclopentyloxy-4-methoxyphenyl)cyclopentane-1-carboxylate]	2.7	0.021	0.0078
N-[2-(3-cyclopentyloxy-4-methoxyphenyl)ethyl]oxamide	0.89	0.012	0.013
R-rolipram	0.31	0.004	0.013
N-[2-(3,4-bisdifluoromethoxy-phenyl)ethyl]oxamide	1.6	0.04	0.25
Ro 20-1724	2.6	0.095	0.07

TABLE I-continued

Compound	Low Affinity IC_{50}	High Affinity IC_{50}	High/Low Ratio
S-rolipram	1.1	0.95	0.07
(R)-(+)-1-(4-bromobenzyl)-4-[(3-cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone	4.0	0.45	0.11
1-(4-aminobenzyl)-4-(3-cyclopentyloxy-4-methoxyphenyl)-2-imidazolidinone	1.4	0.01	0.07
denbufylline	0.29	0.05	0.17
3-(cyclopentyloxy-4-methoxyphenyl)-1-(4-N'-[N2-cyano-S-methyl-isothioureido]benzyl)-2-pyrrolidone	0.1	0.03	0.30
(R)-(+)-1-(4-bromobenzyl)-4-[(3-cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone	0.06	0.02	0.33
IBMX	29.1	20.3	0.698
(S)-(−)-ethyl [4-(3-cyclopentyloxy-4-methoxyphenyl)pyrrolidine-2-ylidene]acetate	0.46	0.45	.98
Papaverine	10	10	1.0
cis-[4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylate]	0.095	0.110	1.1
cis-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-ol]	0.021	0.04	2.0
(R)-(+)-ethyl[4-(3-cyclopentyloxy-4-methoxyphenyl)pyrrolidine-2-ylidene]acetate	0.14	0.3	2.143
2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-one	0.140	0.5	3.571
trequinsin	1.6	5.0	3.125
dipyridamole	5.2	32.5	6.25

Denbufylline is 7-acetonyl,1,3-dibutylxanthine made by SmithKline Beecham. Papaverine is 1 -[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline. Trequinsin is 2,3,6,7-tetrahydro-2-(mesitylimino)-9,10-dimethoxy-3-methyl-4H-primido[6,1-α]isoquinoline-4-one. Dipyrimadole is the generic name for 2,2',2"2'"-[(4,8-dipiperidinopyrimido[5,4-d]pyrimidine-2-6-diyl)dinitrilo] tetraethanol.

These results illustrate that some compounds can selectively inhibit the so called low affinity form as compared with the high affinity form, and vice versa. The significance of this finding is that it is feasible to minimize side effects by designing or choosing compounds which selectively (preferentially) inhibit one site thereby affecting the desired response to the exclusion of another, unwanted, response, or at least to minimize the non-targeted response to a degree where it is not interfering with the intended therapy to an unacceptable degree.

Notwithstanding this work, we have not defined the basis for the disparate SARs for high affinity rolipram binding and low affinity rolipram binding in the PDE IV isozyme,. However it has been discovered that if a compound exhibits an IC_{50} ratio of about 0.1 or greater, calculated as the ratio of the IC_{50} for high affinity rolipram binding form divided by the IC_{50} for the form which binds rolipram with a low affinity, it will have an acceptable therapeutic index. That is, one can now successfully treat a variety of immune and inflammatory diseases while not affecting other physiological phenomena at all or to an unacceptable degree. Herein the most preferred embodiment is inhibiting the low affinity rolipram binding site as a means for treating inflammatory and allergic diseases.

Compounds

This invention covers those compounds which have an IC_{50} ratio (high/low binding) of about 0.1 or greater. This includes any and all compounds which are PDE IV inhibitors as per the test set our herein, and which demonstrate in these, or similar assays, a ratio within the defined range; of particular interest are those compounds which are not in the public domain and/or not tested as or known to be PDE IV inhibitors prior to the filing date of this application.

Examples of compounds which meet the IC_{50} ratio standard are given in Table 1 above as well as pending U.S. patent applications U.S. Ser. No. 862,083 filed Oct. 30, 1992; U.S. Ser. No. 862,111 filed Oct. 30, 1992; U.S. Ser. No. 862,030 filed Oct. 30, 1992; and U.S. Ser. No. 862,114 filed Oct. 30, 1992. Each of these applications is incorporated herein by reference in full as if set out in this document.

Preferred compounds of this invention are those which have an IC_{50} ratio of greater than 0.5, and particularly those compounds having a ratio of greater than 1.0. Preferred compounds are trequinsin, dipyridamole, and papaverine. Compounds such as cis-[cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylate], 2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-one, and cis-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl) cyclohexan-1-ol] are examples of structures which bind preferentially to the low affinity binding site and which have an IC_{50} ratio of 0.1 or greater.

The following examples are provided to illustrate how to make and use the invention.

They are not in any way intended to limit the scope of the invention in any manner or to any degree. Please refer to the claims for what is reserved to the inventors hereunder.

EXAMPLES

Eight different assays spread among five species were used to develop data supporting the selection of an IC_{50} ratio of about 0.1 or greater. The assays were: stimulation of acid production from rabbit isolated parietal gland; inhibition of FMLP-induced degranulation (release of myleoperoxidase) in human neutrophils; inhibition of FMLP-included O_2^- formation in guinea pig eosinophils; inhibition of LPS-induced TNF_α production in human monocytes; production of emesis in dogs; inhibition of antigen-induced bronchoconstriction in guinea pigs; reversal of reserpine-induced hypothermia in mice; and inhibition of LPS-induced TNF_α production from adoptively-transferred human monocytes in mice. These assays and data are presented below.

Statistical Analysis

To examine the hypothesis that inhibition of the low affinity site PDE IV is associated with the anti-inflammatory actions of this class of compounds, whereas inhibition of the high affinity site is associated with the production of certain side effects, we determined the ability of various PDE IV inhibitors to block inflammatory cell function both in vitro and in vivo and the ability of these compounds to produce side effects in in vitro and in vivo models. To compare the ability of PDE IV inhibitors to elicit a given therapeutic effect or side effect with their ability to inhibit the low affinity binding site versus their ability to inhibit the high affinity site of PDE IV, we compared the potency of these compounds in the in vitro or in vivo assays with their potency against the isolated enzyme catalytic activity or the high affinity site by a linear correlation of (r^2) or a rank order correlation (Spearman's Rho). The linear correlation asks whether the potency of a compound at inhibiting either the low affinity site or the high affinity site can be used to predict the ability to elicit a given anti-inflammatory or side effect.

The rank order correlation tests whether the rank order potency in producing a given anti-inflammatory or side effect is similar to the rank order potency in inhibiting the low affinity or the high affinity site. Both r^2 and Spearman's Rho were calculated using the STAT View II computer program for the Macintosh.

PDE IV versus Rolipram High Affinity Binding

Example 1

Phosphodiesterase and Rolipram Binding Assays

Isolated human monocyte PDE IV and HPDE IV was determined to exist primarily in the low affinity form. Hence, the activity of test compounds against the low affinity form of PDE IV can be assessed using standard assays for PDE IV catalytic activity employing 1 μM [^{3}H]cAMP as a substrate (Torphy et al., 1992).

Rat brain high speed supernatants were used as a source of protein and both enantionmers of [^{3}H]-rolipram were prepared to a specific activity of 25.6 Ci/mmol. Standard assay conditions were modified from the published procedure to be identical to the PDE assay conditions, except for the last of the cAMP: 50 mM Tris HCl (pH 7.5), 5 mM $MgCl_2$, 50 μM 5'-AMP and 1 nM of [^{3}H]-rolipram (Torphy et al., 1992). The assay was run for 1 hour at 30° C. The reaction was terminated and bound ligand was separated from free ligand using a Brandel cell harvester. Competition for the high affinity binding site was assessed under conditions that were identical to those used for measuring low affinity PDE activity, expect that [^{3}H]-cAMP was not present.

Example 2

Aminopyrine Accumulation

Certain methylxanthines and other non-selective PDE inhibitors increase acid secretion in a variety of species. Certain selective PDE IV inhibitors, e.g., rolipram and Ro 20-1724, enhance acid secretion in rats, particularly when given in combination with an activator of adenylate cyclase such as histamine. This increase in acid secretion is accompanied by an elevation of histamine-induced cAMP accumulation. This reported information was rested to determine if the phenomena existed. The ability of compounds to induce acid secretion was correlated with their ability against the low affinity site or the high affinity site. The assay used in this work was the accumulation of a weak base, radiolabeled aminopyrine which has been reported to serve as a biochemical marker for increased acid secretion. The assay follows:

Gastric Gland Preparation

Rabbits of either sex were euthanized by cervical dislocation and the stomach removed. The mucosa was dissected from the corpus; the cranial and antral portions of the stomach were discarded. Gastric glands were isolated by a modification of the methods described by Berglindh and Obrink (1976) and Sack and Spenney (1982). The mucosa was then minced and digested with collagenase to isolate the gastric glands. The digested glands were filtered, washed, and resuspended 1:15 (vol:vol) in incubation medium of the following composition: NaCl, 132.4 mM; KCl, 5.4 mM; Na_2HPO_4, 5.0 mM; NaH_2PO_4, 1.0 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.0 mM; $NaHCO_3$, 12.0 mM; rabbit serum albumin, 2 mg/ml; dextrose, 2 mg/ml; at a pH 7.4.

Aminopyrine Accumulation

To determine acid secretion, the gastric glands in combination with [^{14}C]-aminopyrine, various concentrations of selective PDE IV inhibitors, and a threshold concentration of histamine (0.3–1.0 μM) were incubated at 37° C. on a horizontal shaker (110 cycles/min) for 20 minutes according to the procedures of Sack and Spinney (1982). Samples were then centrifuged and radioactivity in aliquots of the supernatant fraction and pellet were determined. Aminopyrine ratios were calculated as described by Sack and Spenney (1982). The data were expressed as a percent of a response produced by a maximal concentration of histamine (100 μM). EC_{50} values were determined by linear interpolation using the maximum response obtained for each compound.

Example 3

Evaluation of the Emetic Potential of Selective PDE Inhibitors in Dogs

Mongrel dogs (n=5, for each study) of either sex were obtained from the animal colony. After an overnight fast, the dogs were fed ½ can of dog food (Big Bet) at least 30 minutes prior to study. A cannula was placed in the cephalic vein of either foreleg to administer drugs. The cannula was flushed with 1 ml of isotonic saline (0.9%) prior to administration of the experimental compound. Compounds were dissolved in either a mixture of polyethylene glycol and saline or 100% polyethylene glycol and given at a volume of 1.0–2.0 ml/10 kg. To insure that the entire dose entered the circulation, the cannula was flushed with additional 0.5–1.0 ml of saline. The animal was returned to a cage for a 1 hour observation period. Each dog was observed for signs of retching or vomiting and the time after administration of compound for the occurrence of this behavior was noted. At the end of the observation period, the animal was returned to its home cage. Each study day was separated by 7 days. Each compound was administered in ascending doses to each dog on successive study days until an emetic effect was observed. At this time, the individual dog was dropped from the study and higher doses were evaluated in only those dogs that had not yet responded.

The data were expressed as the cumulative percent of dogs responding at each dose as described in the literature for quantal dose response curves. An ED_{50} value was calculated using probit analysis.

Example 4

Guinea Pig Eosinophil Assay

Eosinophil Isolation and Purification

Male (Hartley, Hazelton Labs) guinea pigs were injected with 1 ml of horse serum weekly for 4–6 weeks prior to use. Animals were anesthetized with a mixture of ketamine/xylazine (88 mg; 12 mg/ml; 0.4 ml/kg at least 24 hrs after an injection of horse serum. After the induction of anesthesia the peritoneal cavity was lavaged with 50 ml of warm sterile saline (0.9%). The lavage fluid was collected using a 14 G catheter into 50 ml plastic conical centrifuge tubes. The guinea pigs were allowed to recover from the anesthesia and could be used again after a two-week rest period.

Cells were isolated from the lavage fluid by centrifugation (400×g, 10 min) and were resuspended in 35 ml of phosphate buffered saline (PBS) and underlayed with 10 ml of isotonic Percoll (1.075 g/ml). This suspension was centrifuged for 30 min at 300×g. The pellet containing mainly eosinophils and erythrocytes was washed in PBS and the erythrocytes lysed. These cells were resuspended in RPMI 1640 medium with 20% FBS and incubated overnight at 37° C. in a humidified 5% CO_2 incubator. The next day cells were washed and resuspended in PBS for determination of cell viability (trypan blue exclusion) and purity.

Superoxide Anion Production (O_2^-)

Purified eosinophils (viability>95% and purity>90%) were resuspended in PBS with 20 mM HEPES Buffer (pH 7.4) and 0.1% gelatin at a concentration 1–2×10^6 cell/ml.

Eosinophils (1×10^5) were added to a 96 well plate and were incubated for approximately 1 hr at 37° C. PDE IV inhibitors were added for 10 min prior to the start of the reaction. The reaction was initiated by the addition of cytochrome C (160 μM) and formylMet-Leu-Phe (fMLP) (30 nM) in the absence or presence of 60 units of superoxide dismutase (SOD). Cytochrome C reduction was monitored on a Dynatech MR 7000 plate reader at 550 nm with a 630 nm reference at various time periods. The rate of O_2^- production was determined by linear regression analysis using the net absorbance of wells in the absence or presence of SOD at several time points. Results were expressed as a percent of the control production of O_2^- corrected for basal release. Sine the maximal inhibition observed was 60%, log IC_{30} were calculated using linear interpolation of the concentration and bracketing 30%.

Example 5

Bronchoconstriction in Guinea Pigs

Male Hartley guinea pigs (200–250 g/4 weeks, Hazelton Research, Denver, Pa.) were sensitized by I.M. injections of 0.35 ml of a 5% (w/v) ovalbumin/saline solution into each thigh (0.7 ml total) on Days 1 and 4. Guinea pigs were available for use after day 25.

Experimental Procedure

Male Hartley guinea pigs (600–800 g Hazelton), actively sensitized to ovalbumin, were anesthetized with sodium pentobarbital (40 mg/kg I.P.) approximately 10–15 minutes prior to surgery. The jugular vein, carotid artery, and trachea were cannulated (Deseret Intracath® Vialon® polymer resin radiopaque catheters (Deseret Medical, Inc., Sandy, Utah), 22 GA and 19 GA, and PE tubing 260, respectively) for drug administration, blood pressure monitoring and ventilation. Bilateral vagotomy was performed to minimize cholinergic interference. Animals were paralyzed (pancuronium bromide, 0.1 mg/kg i.v.) and ventilated (45 breaths/min) via a Harvard Rodent Respirator (model 683, Harvard Apparatus, South Natick, Mass.). Airway pressure changes were measured via a side-arm of the tracheal cannula with a Elcomatic transducer (Buxco Electronics, Sharon, Conn.). The ventilatory stroke volume was set to produce a side arm pressure of 8 cm of H_2O (ca 5 cc room air). Blood pressure was measured with a Statham P23XL Physical Pressure Transducer (Viggo-Spectramed, Oxnard, Calif.). Pressures were recorded on a Grass Model 7D Polygraph (Grass Instrument Co., Quincy, Mass.). The animals were kept warm on a heating table throughout the experiment to maintain body temperature.

Test compounds or vehicle were administered via the i.v. route 10 minutes prior to antigen challenge. At the 0 time point, 0.1 mg/kg ovalbumin is administered via the i.v. route. At the peak of the antigen response, an additional dose of antigen, 0.2 mg/kg ovalbumin, i.v. was administered After the peak antigen response to the cumulative 0.3 mg/kg ovalbumin was reached, a saturated KCl solution, 1 cc/kg, i.v., was administered which produced maximal bronchoconstriction.

Example 6

Inhibition of LPS-Induced TNF_α in Human Monocytes

In Vitro Studies

To determine whether TNF_α inhibition is related to inhibition of LPDE IV or HPDE IV, a series of PDE IV inhibitors having a range of potencies for the LPDE IV and HPDE IV were screened for their ability to inhibit TNF_α production in human monocytes stimulated with lipopolysaccharides (LPS) in vitro. The use of primary human cells for this screen was deemed to be extremely important given that different species appear to differ dramatically in the relative contribution of LPDE IV and HPDE IV to cAMP hydrolysis in inflammatory cells.

Methods

TNF_α inhibition was assessed in human peripheral blood monocytes which were purified (Collata) from freshly obtained buffy coats or plasma-phoresis residues of blood from normal human donors. Monocytes were plated at density of 1×10^6 cells/ml medium/well in 24-well multidishes. The cells were allowed to adhere for 1 hr, after which time the supernatant was aspirated and 1 ml of fresh medium (RPMI-1640 containing 1% fetal calf serum and penicillin/streptomycin at 10 U/ml) was added. The cells were incubated for 45 min in the presence or absence of test compounds at concentrations ranging from 1 nM to I mM prior to the addition of LPS (*E. coli.* 055:B5, Sigma Chemicals) to yield a final concentration of 100 ng/ml. Test compounds were solubilized and diluted in a 50:50 concentration of dimethylsulfoxide/ethanol, such that the final solvent concentration in monocyte culture medium was 0.5% dimethylsulfoxide and 0.5% ethanol. Culture supernants were removed from the monocytes after 14–16 hr incubation at 37° C./5%CO_2, and centrifuged at 100xG to remove cell debris. Cytokine assays were performed either immediately or culture supernatants were stored at −70° C. until assayed

Levels of TNF_α were measured using a ELISA (Winston) employing a murine monoclonal anti-human TNF_α antibody (see below) as the capture antibody and a polyclonal rabbit antihuman TNF_α as the second antibody. For detection, a peroxidase-conjugated goat anti-rabbit (Boehringer Mannheim, Cat. #605222) was added followed by a substrate for peroxidase (1 mg/ml orthophenylenediamine with 0.1% urea peroxide). TNF_α levels in samples were calculated from a standard curve generated with recombinant human TNF_α produced in *E. coli.* Monoclonal antibodies to human TNF_α were prepared from spleens of BALB/c mice immunized with recombinant human TNF_α by a modification of the method of Kohler and Millstein (*Nature,* vo. 256, p495–497, 1975). Polyclonal rabbit anti-human TNF_α antibodies were prepared by repeated immunization of New Zealand white rabbits with recombinant human TNF_α emulsified in complete Freund's adjuvant.

In Vivo Suppression of Human TNF_α Production in an Adoptive Peritonitis Model

Methods

One half unit of heparinized venous whole blood was drawn from healthy employees who were not taking any kind of medication. Polymorphonuclear luekocytes were separated by layering the blood on Histopaque-1077 with centrifugation at 800×g for 30 min at 25° C. The lymphocyte/monocyte portion was harvested and washed twice with DPBS (Dulbecco's phosphate buffered saline) without Ca^{2+} and Mg^{2+} at 1000 rpm for 10 min at 25° C. The pellet was resuspended in 5 ml of DPBS without Ca^{2+} and Mg^{2+}, layered on 5 ml Percoll solution prepared in RPMI 1640 medium which was devoid of serum at 25° C., and centrifuged at 550×g for 30 min at 25° C. The buoyant layer of monocytes was removed and washed twice with DPBS without Ca^{2+} and Mg^{2+} at 1000 rpm for 10 min at 25° C. The final washed monocyte isolate was suspended at $6\text{–}10\times10^6$ cells/ml in DPBS without Ca^{2+} and Mg^{2+} at 25° C. Monocytes were also isolated by the same procedure from Source Leukocytes packs. The monocyte preparations ranged from 65 to 90% monocytes and the viability of the cells was >97% (trypan blue exclusion).

BALB/c male (Charles River Laboratories, Wilmington, Mass.) in groups of 4 or 5, were maintained in a barriersustained facility. Mice weighting 18–25 g and of the same age were injected with 0.5 ml of 6–10×10^6 monocytes/ml into the peritoneum using light pressure on a syringe with a 23 ga needle so that the monocytes were exposed to minimal shearing forces and stress. Within 2 min of receiving monocytes, the mice were treated with vehicle or compound by oral dosing for 15 min. The animals were then injected intraperitoneally (i.p.) with 0.2 ml of 125 mg/ml of endotoxin (*E. coli.*, type W, Difco) dissolved in DPBS without Ca^{2+} and Mg^2+. Two hr later, the animals were euthanized by carbon dioxide asphyxiation and 1.5 ml of DPBS without Ca^{2+} and Mg^{2+} (4° C.) was injected i.p. The peritoneum was gently massaged and the wash was removed and placed in polyproylene tubes in an ice bath. The samples were clarified by centrifugation (12,500xg for 5 min at 4° C.). The supernatants were decanted into new tubes (may be stored at −20° C.) and assayed for human and mouse TNF_α by ELISAs. ED_{50} values were calculated by standard procedures.

Example 7

Human Neutrophils Methods

Isolation and Purification

Neutrophils (PMNs) were isolated from heparinized blood by gradient centrifugation using Ficoll (Histopaque 1077) followed by dextran sedimentation to remove the erythrocytes. Any remaining erythrocytes were lysed with water for 30 sec and isotonicity restored using 10×DB-PBS (w/o Ca^{2+} or Mg^{2+}). PMNs were isolated by centrifugation and were washed one additional time with 1×DB-PBS prior to determining cell number and viability (trypan blue dye exclusion). Cell number was adjusted to 0.75–1.5×10^6 cells/ml depending on the individual donor.

Degranulation (Release of Myleoperoxidase)

An aliquot (0.1 ml) of the above cell suspension was incubated in Earles Balanced Salt Solution containing 20 mM HEPES buffer (pH=7.4) and 0.1% gelatin in the presence of 5 μg/ml of cytochalasin B for 5 min at 37° C. in a shaking water bath. Cells were pretreated for additional 5 min with various concentrations of selective PDE IV inhibitors and PGE_2 (3–10 nM) prior to addition of fMLP (30 nM). fMLP was added and the incubation continued for an additional 30 min. The reaction was terminated by placement of the samples on ice followed by centrifugation. The supernatant fraction was removed and stored frozen (−30° C.) until assay for myeloperoxidase activity.

Determination of Myleoperoxidase Activity

Myeloperoxidase activity was determined using o-dianisidine as substrate and horseradish peroxidase as a standard. An aliquot (50 μl) of supernatant was incubated with 100 μl of substrate (o-dianisidine, 0.53 mM; H_2O_2, 0.147 mM; final concentration) in 50 mM Na Phosphate buffer (pH 6.0). The reaction was terminated by the addition of 50 μL of 4 M H_2SO_4. Product formation was determined by measuring absorbance at 410 nm and activity determined by comparison to the standard curve using horseradish peroxidase. Data were expressed as percent of control (amount of myeloperoxidase released in the presence of PGE_2 alone). Since the maximal inhibition observed for the majority of compounds was 30%, log (IC_{15}) values were calculated using linear interpolation of the concentrations bracketing 15%.

Example 8

Reversal of Reserpine-Induced Hypothermia in Mice

Male CF-1 or BALB/c mice were individually isolated in wire cages. The rectal temperature of each mouse was recorded prior to pretreatment with reserpine (10 mg/kg, i.p.). Four hours after reserpine the rectal temperatures were recorded and individual animals were given various doses (orally) of either test compounds, vehicle, or rolipram (10 mg/kg). Rectal temperatures were then recorded every 30 min for 2 hr. The data were expressed as the change in temperature from that observed at four hrs post reserpine (temperatures dropped approximately 10–15° C. below basal levels). Dose-response curves were constructed using temperature changes recorded at 90 or 120 min after treatment. ED_{50} values were determined by probit analysis or linear regression of the means of 6–9 animals. To compare the ability of compounds to reverse reserpine-induced hypothermia with their ability to inhibit low affinity binding or high affinity binding, the ED_{50} and IC_{50} values were expressed as −log (value).

Example 9

Relationship Between Biological Function and Inhibition of PDE IV

To determine if certain biological effects of PDE IV inhibition were associated with inhibition of either the LPDE IV or HPDE IV a comparison between the ability of compounds to produce an effect and the ability of compounds to inhibit LPDE IV or HPDE IV was determined using a linear and rank order correlation. These correlations can be influenced by several factors: 1) the stability of compounds; 2) ability of compounds to enter cells; 3) in in vivo studies, the bioavailability of compounds; 4) the correlation values, especially the linear correlation are sensitive to the difference in potencies, the greater the range of potency values the easier it is to measure a significant linear correlation. These caveats were taken into consideration when analyzing and summarizing the correlation between inhibition of LPDE IV or HPDE IV and the biological function in the various assay systems.

Using isolated inflammatory cells, suppression of monocyte TNF_α production and inhibition of superoxide production in guinea pig eosinophils was better correlated with inhibition of LPDE IV and not HPDE IV. Furthermore, prevention of antigen-induced bronchoconstriction in vivo was better correlated with inhibition of LPDE TV than HPDE IV. In this in vivo model, PDE IV inhibitors appear to act by preventing mast cell degranulation (Underwood et al., in press). However, inhibition of inflammatory cell function was not always associated with inhibition of LPDE IV because it was found that inhibition of neutrophil degranulation was better correlated with inhibition of HPDE IV than LPDE IV. Thus it appears that some but not all suppression of inflammatory cell activity was associated with inhibition of LPDE IV. In contrast, enhancement of acid secretion, production of emesis and reversal of resperpine-induced hypothermia (a measure of the psychtropic potential of PDE IV inhibitors) were better correlated with inhibition of HPDE IV and not LPDE IV. Thus most of the potential side effects of this class of compounds were associated with inhibition of HPDE IV.

Thus these findings suggest that compounds which preferentially inhibit LPDE IV will produce beneficial anti-inflammatory effects with reduced potential to elicit unwanted side effects. Thus selecting compounds with an IC_{50} ratio of about 0.1 or greater as regards the IC_{50} for PDE IV catalytic form which binds rolipram with a high affinity divided by the IC_{50} for PDE IV catalytic form which binds rolipram with a low affinity (HPDE IV/LPDE IV) should result in an increase in their therapeutic index, i.e., the salutory effect is maximized and the deleterious effect is minimized.

To determine if this selection guide would indeed identify compounds with an improved therapeutic index, three models comparing a therapeutic effect with a side effect were evaluated These included an in vitro comparison between the ability of compounds to suppress TNFα production from isolated human monocytes with their ability to stimulate acid secretion in isolated rabbit parietal glands and two in vivo comparisons examining the ability of compounds to prevent antigen-induced bronchoconstriction in guinea pigs and the ability to elicit emesis in dogs and the ability of compounds to suppress TNFα production in an adoptive transfer model in mice and their ability to reverse reserpine-induced hypothermia in mice.

PDE IV inhibitors with a selectivity ratio (HPDE IV/LPDE IV) of equal to or greater than 0.1 showed a marked improvement in their therapeutic index. For example, cis-[4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylate], 2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-one, and cis-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl) cyclohexan-1-ol] all with selecitivy ratio of $\geqq 0.1$ demonstrate a 100×fold improvement in their therapeutic index in comparison with the archetypal PDE IV inhibitor, R-rolipram. Thus, this demonstrates that using the selection guide of HPDE IV IC_{50}/LPDE IV $IC_{50} \geqq /0.1$ identifies compounds with an increased therapeutic index in vitro comparison.

We claim:

1. A method for identifying a PDE IV inhibitor useful for treating inflammation while minimizing gastrointestinal and psychotropic effects, which method comprises determining for said inhibitor the IC_{50} for its binding with the PDE IV form which binds rolipram with a high affinity and its IC_{50} for its binding with the PDE IV form which binds rolipram with a low affinity, and determining whether the ratio of high affinity to low affinity IC_{50}s is about 0.1 or greater.

2. The method of claim **1** wherein the method identifies an inhibitor which has an IC_{50} ratio of about 0.1 or greater as determined by using rat brain PDE IV as the form which binds rolipram with a high affinity and human PDE IV as the form which binds rolipram with a low affinity.

3. The method of claim **1** wherein the low affinity binding form of PDE IV is derived from human monocytes.

4. The method of claim **1** wherein the low affinity binding form of PDE IV is a recombinant human PDE IV.

5. The method of claim **1** wherein the PDE IV is from a tissue cellular or recombinant source.

6. The method of claim **1** wherein the PDE IV used as the high affinity binding form of PDE IV is the high-speed supernatant of a rat brain cell homogenate.

7. The method of claim **1** wherein the PDE IV is a recombinant protein.

8. The method of claim **1**, **2**, **3**, **4**, **5**, **6** or **7** wherein the IC_{50} ratio is about 0.5 or greater.

9. The method of claim **1**, **2**, **3**, **4**, **5**, **6** or **7** wherein the IC_{50} ratio is about 1.0 or greater.

10. A method for identifying a PDE IV inhibitor useful as a bronchodilator while minimizing gastrointestinal and psychotropic effects, which method comprises determining for said inhibitor the IC_{50} for its binding with the PDE IV form which binds rolipram with a high affinity and its IC_{50} for its binding with the PDE IV form which binds rolipram with a low affinity, and determining whether the ratio of high affinity to low affinity IC_{50}s is about 0.1 or greater.

11. The method of claim **10** wherein the PDE IV is from a tissue cellular or recombinant source.

12. The method of claim **10** wherein the method identifies an inhibitor which has an IC_{50} ratio of about 0.1 or greater as determined by using rat brain homogenate as the source of PDE IV for the form which binds rolipram with a high affinity and a human monocytes homogenate as the source of PDE IV for the form which binds rolipram with a low affinity.

13. The method of claim **10** wherein the PDE IV is a recombinant human PDE IV.

14. The method of claim **10** wherein the PDE IV used as the high affinity binding form of PDE IV is derived from the high-speed supernatant of a rat brain cell homogenate.

15. The method of claim **10** wherein the PDE IV is a recombinant protein.

16. The method of claim **10**, **11**, **12**, **13**, **14**, or **15** wherein the IC_{50} ratio is about 0.5 or greater.

17. The method of claim **10**, **11**, **12**, **13**, **14**, or **15** wherein the IC_{50} ratio is about 1.0 or greater.

* * * * *

Exhibit D

US005716954A

United States Patent [19]

Wilhelm et al.

[11] **Patent Number: 5,716,954**

[45] **Date of Patent: Feb. 10, 1998**

[54] **BENZOPYRIDAZINONE AND PYRIDOPYRIDAZINONE COMPOUNDS**

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[73] Assignee: **Syntex U.S.A. Inc.**, Palo Alto, Calif.

[21] Appl. No.: **369,041**

[22] Filed: **Jan. 5, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 86,954, Jul. 2, 1993, abandoned, which is a continuation-in-part of Ser. No. 775,679, Oct. 9, 1991, abandoned, and PCT/US92/0398, Oct. 8, 1992.

[51] **Int. Cl.^{6}** **A61K 31/535**; A61K 31/50; C07D 471/04; C07D 237/32

[52] **U.S. Cl.** **514/234.2**; 514/248; 544/117; 544/236; 544/237; 546/322; 546/326; 560/52

[58] **Field of Search** 544/236, 117; 514/248, 234.2

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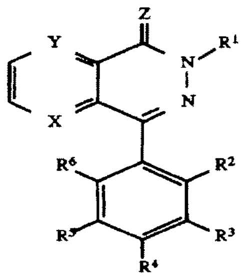
Primary Examiner—Emily Bernhardt
Attorney, Agent, or Firm—Heller Ehrman White & McAuliffe

[57] **ABSTRACT**

Benzo or pyridopyridazinones and pyridazinthiones of the formula

Formula I



wherein:

X and Y are nitrogen or carbon, provided that at least one is carbon, and Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl;

R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower-alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro;

or a pharmaceutically acceptable ester, ether or salt thereof, have been found to be useful as an anti-inflammatory, antasthmatic, immunosuppressive, anti-allograft rejection, anti-graft-vs-host rejection, autoimmune disease or analgetic agent(s).

45 Claims, No Drawings

BENZOPYRIDAZINONE AND PYRIDOPYRIDAZINONE COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08/086,954, filed Jul. 2, 1993, abandoned. Application Ser. No. 08/086,954 is a continuation-in-part of application Ser. No. 07/775,679, filed Oct. 9, 1991, abandoned, which is incorporated herein by reference. Application Ser. No. 08/086,954 also claims priority from PCT International Application No. PCT/US92/08398, filed Oct. 8, 1992, incorporated herein by reference and itself claiming priority from application Ser. No. 07/775,679.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to benzo and pyridopyridazinones and pyridazinthiones useful as anti-inflammatory agents, antasthmatic agents, immunosuppressive agents, anti-allograft rejection agents, anti-graft-vs-host disease agents, anti-autoimmune agents or analgetic agents, to their intermediates, to their preparation and to pharmaceutical compositions using the compounds of the invention.

2. Background Information

Cyclic 3',5'-adenosine monophosphate (cAMP) modulates a variety of cellular and physiologic functions in mammals, such as cell division, endocrine function, and the immune response. The level of cAMP is controlled, in part, by a class of enzymes called phosphodiesterases, which enzymatically degrade cAMP. There are five families of phosphodiesterase that are categorized according to their function. For instance, phosphodiesterase III (PDE III) is isolated from human platelet cells and modulates platelet aggregation. Another type of phosphodiesterase, (PDE IV), is found in various cells but it is the predominant form in human leukocytes. This enzyme modulates leukocyte activation and function associated with the immune response and inflammation. Both of these phosphodiesterases implement their control by modulating the cellular level of cAMP in their respective cells. Thus, inhibition of phosphodiesterases provides a method of modulating any cellular and bodily function that is controlled by cAMP.

Compounds that are nonspecific phosphodiesterase inhibitors are known, i.e., these compounds inhibit all or multiple types of phosphodiesterases. [See, Beavo, J. A. and D. H. Reifsyder, *Trends in Pharm. Science*, 11:150–155 (1990); and Nicholson, C. D., R. A. J. Challiss and M. Shahid, *Trends in Pharm. Science*, 12:19–27 (1991).]

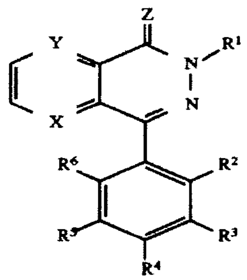
Nonspecific phosphodiesterase inhibitors are of limited value because of numerous side-effects. Since cAMP is involved in so many functions throughout the body, a nonspecific phosphodiesterase inhibitor has the potential to alter all of the functions modulated by cAMP.

It has been surprisingly discovered that certain benzo and pyridopyridazinones are potent selective inhibitors of phosphodiesterase IV (PDE IV). These compounds are well suited for use as a treatment for any disorder in which PDE IV function plays a role, such as where leukocyte activation or function is involved. In particular, these compounds are especially well suited for use as anti-inflammatory agents, antasthmatic agents, immunosuppressive agents, anti-allograft rejection agents, anti-graft-vs-host disease agents or anti-autoimmune disease agents.

SUMMARY OF THE INVENTION

One aspect of the present invention relates to benzo or pyridopyridazinones and pyridazinthiones, i.e., the compounds of Formula I:

Formula I



wherein:

X and Y are nitrogen or carbon, provided that at least one is carbon, and Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl;

R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower-alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro; or a pharmaceutically acceptable ester, ether or salt thereof.

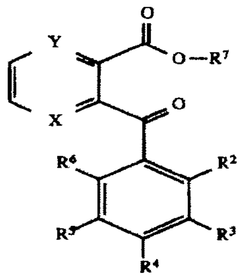
In another aspect, the invention relates to a pharmaceutical composition containing a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable ester or salt thereof admixed with at least one pharmaceutically acceptable excipient.

In still another aspect, the invention relates to a method of use as an anti-inflammatory, antasthmatic, immunosuppressive, anti-allograft rejection, anti-graft-vs-host rejection, autoimmune disease, or analgetic agent, by administering to a mammal in need of such treatment a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable ester or salt thereof.

Yet another aspect of the invention relates to the treatment of the above conditions or diseases by the selective inhibition of phosphodiesterase (PDE) IV.

Yet another aspect of the invention relates to precursors for making the compounds of Formula I and the pharmaceutically acceptable salts and esters thereof, represented by Formula II:

Formula II



wherein:

X and Y are nitrogen or carbon atoms, provided that at least one is carbon;

R^2, R^3, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower-alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro;

R^4 is hydrogen, lower alkyl, iodo, bromo, chloro, carboxy, esters, carbamoyl, lower-alkyl carbonyl, halocarbonyl or nitro; and

R^7 is hydrogen or lower alkyl;

or a pharmaceutically acceptable ester, ether or salt thereof.

Another aspect of the invention relates to processes for making the compounds of Formula I and the pharmaceutically acceptable salts and esters thereof. For example, a compound of Formula I (where Z is oxygen) is made by reacting a compound of Formula II with a suitably substituted hydrazine. Or, a compound of Formula I (where Z is oxygen) is treated with a thiation reagent for ketones, e.g., Lawesson's Reagent, to give the corresponding compound of Formula I where Z is sulfur.

Another aspect of the invention relates to processes for making the compounds of Formula I (where R^1 is lower alkyl, aryl or heteroaralkyl) by reacting a compound of Formula I (where R^1 is hydrogen) with a suitably substituted alcohol.

In another aspect, this invention provides compositions useful in the treatment of inflammatory, asthmatic, allograft rejection, graft-vs-host or autoimmune conditions or diseases, or pain in mammals through its use as an anti-inflammatory, antasthmatic, immunosuppressive, anti-allograft rejection, anti-graft-vs-host rejection, autoimmune disease, or analgetic agent, wherein the composition comprises a therapeutically effective amount of a compound of Formula I as described above and a pharmaceutically acceptable excipient.

DETAILED DESCRIPTION

Definitions and General Parameters

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The term "alkyl" refers to a branched or straight chain monovalent hydrocarbon radical of one to twenty-four carbon atoms.

The term "lower alkyl" refers to a branched or straight chain saturated hydrocarbon radical of one to nine carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, isopropyl, i-butyl, n-butyl, t-butyl, pentyl, n-hexyl, n-heptyl.

The term "lower alkoxy" refers to the group —O—R' where R' is lower alkyl.

The term "cycloalkyl" refers to a monovalent carbocyclic hudrocarbon radical of three to seven carbon atoms. The term is exemplified by such radicals as cyclopropyl, cyclopentyl, cyclohexyl and cycloheptyl.

The term "halo" refers to fluoro, bromo, chloro, iodo.

The term "carboxy" refers to the group —C(O)OH.

The term "alkoxycarbonyl" refers to the group —C(O)OR where R is alkyl.

The term "lower-alkyl carbonyl" refers to the group —C(O)—(lower-alkyl)

The term "halocarbonyl" refers to the group —C(O)X where X is halo.

The term "carbamoyl" refers to the group —C(O)NR'R" where R' and R" are independently hydrogen or lower alkyl.

The term "aryl" refers to a monovalent unsaturated aromatic carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl), which can optionally be mono-, di-, tri-, tetra- or penta-substituted, independently, with hydroxy (phenol), lower alkyl (toluenyl or benzyl), lower alkoxy, chloro, fluoro, trifluoromethyl, carbamoyl, nitro and/or cyano.

The term "aralkyl" refers to the group aryl-(lower alkyl), wherein aryl and lower alkyl are as defined above. Typical aralkyl groups are, e.g. benzyl (i.e., phenytmethyl), 4-methoxybenzyl, 4-chlorobenzyl, 3,5-dichlorobenzyl, 2-naphthylmethyl and the like.

The term "heterocyclo" refers to saturated or unsaturated monovalent mono- or poly- carbocyclic radicals having at least one hetero atom (such as nitrogen, oxygen or sulfur) or a combination thereof, which can optionally be substituted, independently, with, e.g., hydroxy, amino, imino, lower alkyl, lower alkoxy, carboxy, carbamoyl, lower-alkyl carbonyl, halocarbonyl, aryl, aralkyl, halo, cyano, heteroaryl and/or heterocyclo. Further, the term also includes instances where a heteroatom of the radical has been oxidized, e.g., N-oxides, sulfoxides, sulfones, or oxo. For example, typical heterocyclo groups with one or more nitrogen or sulfur atoms are pyrrolinyl, pyrrolidinyl, dioxolanyl, imidazolinyl, imidazolidinyl, pyrazolinyl, pyrazolidinyl, pyranyl, piperidinyl, dioxoanyl, morpholinyl, 1,4-dithianyl, thiomorpholinyl, piperazinyl, 1,3,5-trithianyl, and the like.

The term "heterocyclo lower-alkyl" refers to the group heterocyclo-(lower alkyl), wherein heterocyclo and lower alkyl are as defined above. Typical heterocyclo lower-alkyl groups are, e.g. piperidinylmethyl, 2-norboranylmethyl, 2-(1,3-dioxolanyl)methyl, 3-(1-methylpyridonyl)methyl, 8-(6-fluorobenzo-1,3-dioxanyl)methyl and the like.

The term "heteroaryl" refers to aromatic monovalent mono- or poly- carbocyclic radicals having at least one hetero atom (such as nitrogen, oxygen or sulfur) or a combination thereof, which can optionally be substituted, independently, with, e.g., hydroxy, amino, imino, lower alkyl, lower alkoxy, carboxy, carbamoyl, lower-alkyl carbonyl, halocarbonyl, aryl, aralkyl, halo, cyano, heteroaryl, and/or heterocyclo. Further, the term also includes instances where an atom of the radical has been oxidized, e.g., N-oxides, sulfoxides, sulfones, or oxo. For example, typical heteroaryl moieties with one or more nitrogen or sulfur atoms are pyrroyl, pyridinyl, furyl, thienyl, [oxazoyl, thiazoyl, imidazoyl, pyrazoyl, isoxazolyl, isothiazoyl, 1,2,3-oxadiazoyl, 1,2,3-triazoyl, 1,3,4-thiadiazoyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, indoyl, benzo[b]furanyl, benzo[b]thiophenyl, benzimidazoyl, benzthiazoyl, purinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl and the like.

The term "heteroaralkyl" refers to the group heteroaryl-(lower alkyl), wherein heteroaryl and lower alkyl are as defined above. Typical heteroaralkyl groups are, e.g. 4-pyridylmethyl, 3-pyridylmethyl, 2-pyridylmethyl, 2-pyridylpropyl, 3-thienylmethyl, 2-thienylethyl, 2-imidazoyl, 3,5-dimethylisoxazolylmethyl, 3-pyridonylmethyl, 5-(4-methylthiazolyl)methyl, 3-furanylmethyl, 2-furanylmethyl, 5-(2-methyl-5-nitroimidazolyl)methyl, 4-[2-(4-chlorophenyl)thiazolyl]methyl, 4-(2-methylthiazolyl)methyl, 4-(5-phenyl-1,2,4-oxadiazolyl)methyl, 4-[2-(4-methoxybenzyl)thiazolyl]methyl, 3-(1,2,4-oxadiazolyl)methyl, 3-[5-(3,5-dimethyl-isoazoyl-4-yl)-1,2,4-oxadiazolyl]methyl and the like.

The term "N-oxide" refers to nitrogen heterocycles where a nitrogen atom in the ring has been oxidized, e.g., 4-pyridyl-N-oxide, 3-pyridyl-N-oxide, or 2-pyridyl-N-oxide.

The term "esterification reagent" refers to a reagent (e.g., diazomethane, methanol, methyl iodide, ethyl iodide or ethanol) that when contacted with a carboxy group results in the formation of the corresponding alkoxycarbonyl group.

The term "alkylating agent" refers to a halo-substituted alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl compound (e.g., iodoethane, 2-iodopropane, iodobutane, iodopentane, 4-picolyl chloride, 3-picolyl chloride or benzyl bromide) that, when in the presence of a base, can join the alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl moiety to a nitrogen atom.

The term "compound", as used in the detailed description in reference to the compound of Formula I, is intended to refer to the pharmaceutically acceptable salts, esters and ethers of the compound, unless expressly stated otherwise, such as "the compound of Formula I as a free base".

The term "pharmaceutically acceptable salt" refers to any salt derived from an inorganic or organic acid or base. The term "pharmaceutically acceptable anion" refers to the anion of such acid addition salts. The term "pharmaceutically acceptable cation" refers to the cation of such base addition salts. The salt, anion and/or the cation are chosen not to be biologically or otherwise undesirable.

The anions are derived from inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid (giving the sulfate and bisulfate salts), nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, hexanoic acid, heptanoic acid, cyclopentanepropionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, o-(4-hydroxybenzoyl)- benzoic acid, benzenesulfonic acid, p-chlorobenzene- sulfonic acid, 2-naphthalenesulfonic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane disulfonic acid, 2-hydroxyethanesulfonic acid, salicylic acid, p-toluensulfonic acid and the like.

The cations are derived from bases, such as alkaline earth hydroxides, including calcium hydroxide, potassium hydroxide, potassium carbonate, sodium hydroxide, sodium hydride, lithium hydroxide and the like, preferably sodium hydroxide.

The term "pharmaceutically acceptable esters" and "pharmaceutically acceptable ethers" refer to those compounds of Formula I where an oxygen or a nitrogen has been modified, e.g., acylated by the addition of the group —C(=O)—W, wherein W is an alkyl group containing 1 to 20 carbon atoms including adamantyl, aryl, aralkyl, amino, alkylamino, dialkylamino, an alkoxy group containing 1 to 20 carbon atoms, $—CH_2—O—CH_3$, or $—CH_2—NH_2$. This invention contemplates those compounds of Formula I which are esters as described herein and at the same time are the pharmaceutically acceptable acid addition salts thereof. The terms "inert organic solvent" or "inert solvent" refer to a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform, methylene chloride (or dichloromethane), diethyl ether, pyridine and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert organic solvents.

As used herein, the term "autoimmune disease" refers to disorders wherein the immune system of a mammal mounts a humoral or cellular immune response to the mammal's own tissue or to antigenic agents that are not intrinsically harmful to the mammal, thereby producing tissue injury in such a mammal. Examples of such disorders include, but are not limited to, systemic lupus erythematosus, rheumatoid arthritis and type I diabetes.

As used herein, the term "allograft rejection" refers to the humoral or cellular immune response mounted by the immune system of a mammal after it has received a histo-incompatible tissue graft from another mammal of the same species, thereby producing tissue injury in such a mammal.

As used herein, the term "graft-vs-host disease" refers to the immune response that originates from transplanted graft tissue, in particular, transplanted bone-marrow tissue, and that is directed towards the host tissue, thereby producing tissue injury in the host.

As used herein, the term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the condition or disease, that is, avoiding any clinical symptoms of the disease;

(ii) inhibiting the condition or disease, that is, arresting the development or progression of clinical symptoms; and/or

(iii) relieving the condition or disease, that is, causing the regression of clinical symptoms.

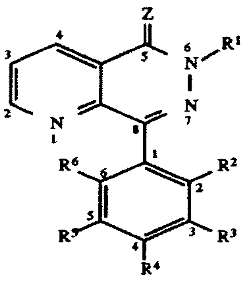
As used herein, the term "therapeutically effective amount" refers to that amount of a compound of Formula I which, when administered to a mammal in need thereof, is sufficient to effect treatment (as defined above) as an anti-inflammatory, antasthmatic, immunosuppressive, anti-allograft rejection, anti-graft-vs-host disease, autoimmune disease or analgetic agent(s). What amount constitutes a "therapeutically effective amount" will vary depending on the compound, the condition and its severity, and the mammal to be treated, but may be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

As used herein, the term "q.s." means adding a quantity sufficient to achieve a stated function, e.g., to bring a solution to a desired volume (e.g., 100 mL).

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure over a temperature range from about −100° C. to about 200° C., more preferably from about 10° C. to about 50° C., and most preferably at about room (or "ambient") temperature, e.g., about 20° C.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, preparative high pressure liquid chromatography (preparative HPLC), thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

For the compounds of the instant invention containing a pyridopyridazinone ring moiety, where X is nitrogen, Y is carbon, and Z is oxygen (i.e., pyrido[2,3-d]pyridazin-5-one compounds), the following numbering system will be used for naming said compounds.



Formula I

Some representative compounds are named in the following examples.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be named 6-(4-pyridylmethyl)-8-phenyl-pyrido[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be named 6-benzyl-8-phenyl-pyrido-[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-benzyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-benzyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^5 is bromo, R^2, R^4 and R^6 are hydrogen, can be named 6-benzyl-8-(3-chloro-5-bromophenyl)-pyrido[2,3-d]pyridazin-5-one.

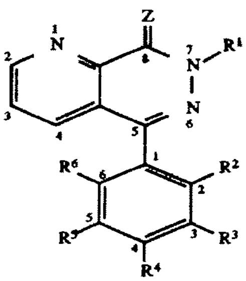
The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is ethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-ethyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one.

The compound of Formula I where X is nitrogen, Y is carbon, Z is sulfur, R^1 is benzyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 6-benzyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-thione.

For the compounds of the instant invention containing a pyridopyridazinone ring moiety, where X is carbon, Y is nitrogen, and Z is oxygen (i.e., pyrido[2,3-d]pyridazin-8-one compounds), the following numbering system will be used for naming said compounds.



Formula I

Some representative compounds are named in the following examples.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 4-pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be named 7-(4-pyridylmethyl)-5-phenyl-pyrido[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be named 7-benzyl-5-phenyl-pyrido-[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 7-benzyl-5-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 7-benzyl-5-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-8-one.

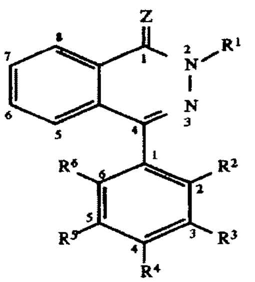
The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^5 is bromo, R^2, R^4 and R^6 are hydrogen, can be named 7-benzyl-5-(3-chloro-5-bromophenyl)-pyrido[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is ethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 7-ethyl-5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 7-(4-pyridyl-N-oxide-methyl)-5-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-8-one.

The compound of Formula I where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 7-(4-pyridyl-N-oxide-methyl)-5-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-8-one.

For the compounds of the instant invention containing a benzopyridazinone ring moiety (e.g., the compounds of Formula I where X and Y are carbon atoms, and Z is oxygen) the following numbering system will be used for naming said compounds.



Formula I

Some representative compounds are named in the following examples.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is 4-pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be named 4-phenyl-2-(4-pyridylmethyl)-1-(2H) phthalazinone.

The compound of Formula I where X and Y are carbon, R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, can be n is oxygen, amed 4-phenyl-2-benzyl-1-(2H) phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is benzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 4-(3-nitrophenyl)-2-benzyl-1-(2H) phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 4-(3-chlorophenyl)-2-benzyl-1-(2H)phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is benzyl, R^3 is chloro, R^5 is bromo, R^2, R^4 and R^6 are hydrogen, can be named 4-(3-chloro-5-bromophenyl)-2-benzyl-1-(2H) phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is ethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 4-(3-chlorophenyl)-2-ethyl-1-(2H) phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 4-(3-nitrophenyl)-2-(4-pyridyl-N-oxide-methyl)-1-(2 H)phthalazinone.

The compound of Formula I where X and Y are carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, can be named 4-(3-chlorophenyl)-2-(4-pyridyl-N-oxide-methyl)-1-(2H) phthalazinone.

SYNTHESIS OF THE COMPOUNDS OF FORMULAE I AND II

The compounds of Formulae I and II are synthesized as described with reference to Reaction Scheme A. As used in Reaction Scheme A, X and Y are nitrogen or carbon atoms, provided at least one is a carbon atom, and Z is oxygen. R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl; and R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower-alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro.

Reaction Scheme A illustrates the preparation of novel benzo or pyridopyridazinones (Formula I where X is nitrogen, Y is carbon, and Z is oxygen) and the corresponding intermediate benzo or pyridoarylketoesters (Formula II).

Reaction Scheme B illustrates a preparation for the intermediate benzo or pyridoarylketoesters (Formula II where X and Y are carbon or nitrogen, provided at least one is carbon) using an organolithium, organocadmium or Grignard reagent.

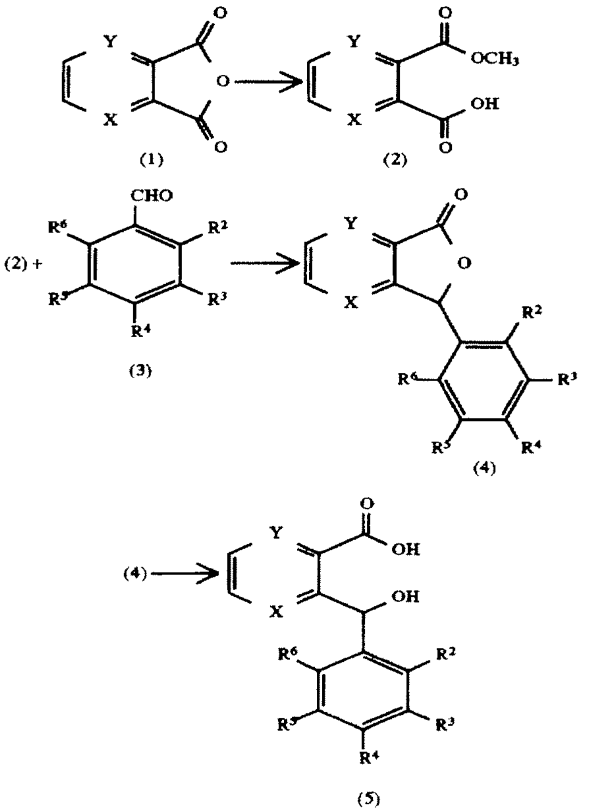
Reaction Scheme C illustrates an alternative preparation of novel benzo or pyridopyridazinones (Formula I where Z is oxygen) from benzo or pyridopyridazinones, where R^1 is hydrogen.

Reaction Scheme D illustrates a preparation for the intermediate benzo or pyridoarylketoesters (Formula II where X and Y are carbon or nitrogen, provided at least one is carbon, and R^3 is nitro).

Reaction Scheme E illustrates a preparation of novel pyridyl-N-oxide-methyl substituted pyridopyridazinones (Formula I) from pyridopyridazinones (Formula I), where R^1 is hydrogen.

Reaction Schemes F and G illustrate an alternative preparation of novel optionally substituted compounds of Formula I, where X is nitrogen, Y is carbon, Z is oxygen, and R^1 is hydrogen.

REACTION SCHEME A



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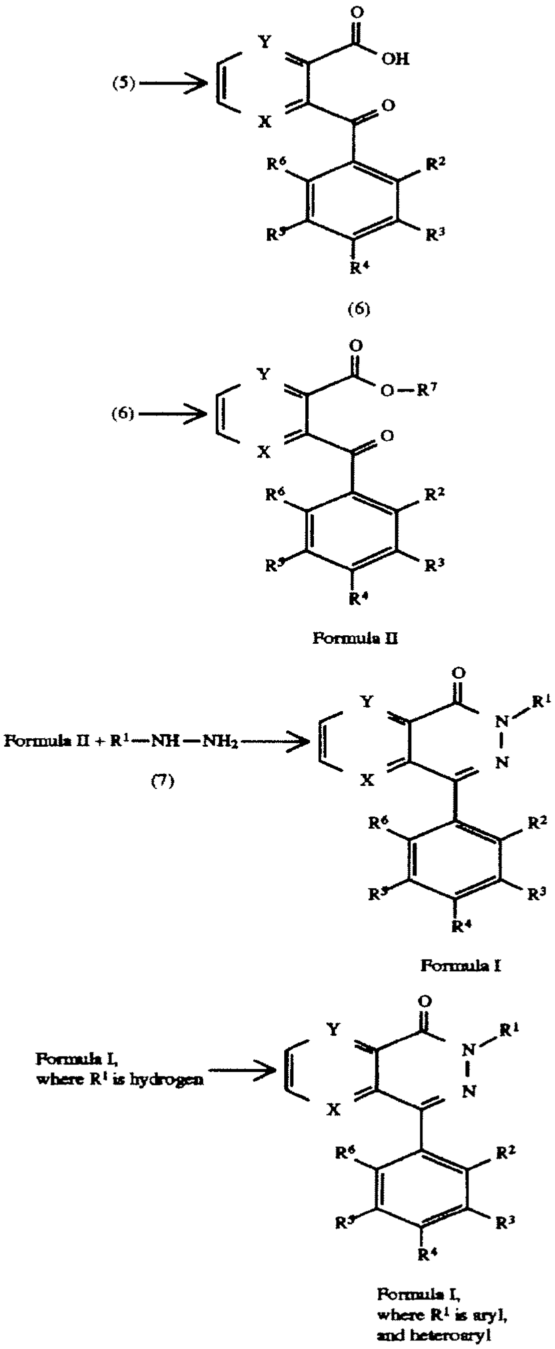
REACTION SCHEME A


(5)
Y
X
O
OH
R6
R2
R5
R3
R4
(6)
O—R7
Formula II
Formula II + R1—NH—NH2
(7)
N
R1
Formula I
Formula I, where R1 is hydrogen
Formula I, where R1 is aryl, and heteroaryl

Starting Materials

Referring to Reaction Scheme A, most of the compounds of Formula 1 are commercially available from Aldrich Chemicals and ICN Chemicals. Similarly, many of the substituted hydrazines (Formula 7) are commercially available from Aldrich Chemicals, those that are not can be prepared by following the disclosures of S. R. Sandler and W. Karo, "Organic Functional Group Preparations" 2nd Ed. 1983, Vol 12-I, Academic Press, New York.

Preparation of Formula 2

A benzo or pyridodicarboxylic anhydride (Formula 1) is dissolved in a solvent [e.g., an alcohol (methanol or ethanol), preferably methanol] and heated for a period of about 2 to 20 minutes, preferably about 10 minutes at a temperature of about 60° to 120° C., preferably about 100° C. The desired product is taken directly to the next step without purification.

Preparation of Formula 4

An optionally substituted benzo or pyridoacidester (Formula 2) is added to about 5.0 molar equivalents of an optionally substituted benzaldehyde (Formula 3) and about 5.0 molar equivalents of a solvent, such as p-cymene, dibromobenzene, nitrotoluene, bromoanisole or xylene, preferably p-cymene. The mixture is heated to a temperature of about 150° C. to 210° C., preferably about 180° C. for a period of about 2 to 6 hours, preferably about 4 hours. The solution is cooled and the excess benzaldehyde and solvent are removed yielding the desired optionally substituted benzo or pyridolactone (the compound of Formula 4). The desired compound can be purified by silica gel chromatography or the like, however, preferably the desired compound is taken without purification directly to the next step.

Preparation of Formula 5

An optionally substituted benzo or pyridolactone (Formula 4) is suspended in a basic solution (e.g., potassium hydroxide, sodium hydroxide or ammonium hydroxide in a solvent such as, methanol or ethanol, preferably potassium hydroxide in methanol at a ratio of about 4.5 grams/100 ml) and refluxed for a period of about 3 to 9 hours, preferably about 6 hours. The mixture is cooled and the solvent removed yielding a benzo or pyridobenzylhydroxy acid (Formula 5).

Preparation of Formula 6

A benzo or pyridobenzylhydroxy acid (Formula 5) is suspended in a solvent that is inert to oxidation agents (e.g., methylene chloride, chloroform or carbon tetrachloride, preferably methylene chloride). To this solution is added about 1.0 molar equivalent of an oxidation agent, such as pyridinium dichromate, pyridinium chlorochromate or manganese dioxide, preferably pyridinium dichromate. The solution is stirred at a temperature in the range of about 0° C. to 50° C., preferably about 21° C. (room temperature) for a period of about 9 to 27 hours, preferably about 18 hours, yielding the desired optionally substituted benzo or pyridobenzoylacid (Formula 6). The desired product can be isolated and purified, however, the product is preferably taken directly to the next step without purification.

Preparation of Formula II

A benzo or pyridobenzoylacid (Formula 6) is dissolved in a solvent (e.g., methylene chloride, chloroform or carbon tetrachloride, preferably methylene chloride) and cooled to a temperature in the range of about −20° C. to 20° C., preferably about 0° C. About 1.2 molar equivalent of an esterification reagent such as, diazomethane, is added to the solution and stirred for a period of about 1 to 5 hours, preferably the reaction is monitored by TLC to completion (about 2 hours). The desired optionally substituted benzo or pyridobenzoylester (Formula II) is isolated and purified by the removal of the solvent followed by chromatography.

Alternatively, an optionally substituted benzo or pyridobenzoylester (Formula II) is prepared by dissolving a suitably substituted benzo or pyridobenzoylacid (Formula 6) in a solvent (e.g., methanol, ethanol or the like, preferably methanol) to which is added about 1 molar equivalent of a strong acid (e.g., concentrated H_2SO_4, HCl (gas), $BF_3.Et_2O$, preferably concentrated H_2SO_4). The solution is stirred for a period of about 6 to 30 hrs, preferably about 18 hrs at the reflux temperature of the solvent used. The solution is cooled and made basic. The desired optionally substituted benzo or pyridobenzoylester (Formula II) is isolated and purified by chromatography.

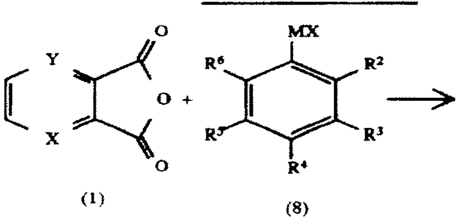
As indicated the desired compound may be purified by silica gel chromatography or the like, however, preferably the desired compound is taken without further purification to the next step.

Preparation of Formula I

An optionally substituted benzo or pyridobenzoylester (Formula II) is suspended in a solvent (about 15 ml/mmole) (e.g., ethanol, methanol or the like, preferably ethanol). To this suspension is added about 2 molar equivalents of an unsubstituted or substituted hydrazine (Formula 7, where R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl). The mixture is refluxed for a period of about 4 hours to 240 hours. The specific time is dependent on the substituent (i.e., R^1) of the hydrazine. Preferably, the reaction mixture is monitored by TLC for completion. The mixture is allowed to cool and filtered to give a crude product. The desired benzo or pyridobenzoylpyridazinones (Formula I) are purified and isolated following extraction and chromatography.

Other optionally substituted compounds of Formula I are prepared by suspending a suitably substituted compound of Formula I, where R^1 is hydrogen, in a solvent (about 10 to 100 ml/mmole). To this suspension is added about 1 to 20 molar equivalents of a suitable base (e.g. sodium hydride, potassium carbonate, potassium iodide) and about 1 to 20 molar equivalents of a suitable alkylating agent (e.g., iodoethane, 4-picolyl chloride, 2-iodopropane). The mixture is refluxed under an inert atmosphere for about 6 to 48 hours, preferably about 18 hours, most preferably the reaction is monitored by TLC for completion. The mixture is allowed to cool and purified following standard chromatographic techniques yielding the desired benzo and pyridobenzoylpyridazinones (Formula I).

REACTION SCHEME B



(1)

(8)

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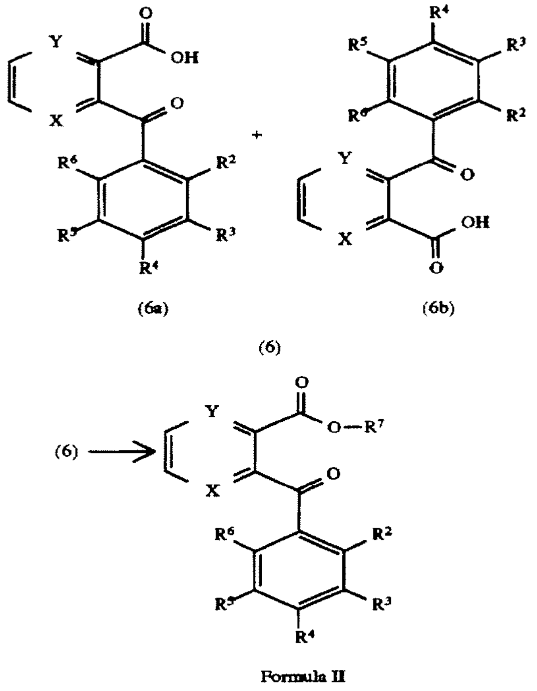
REACTION SCHEME B



(6a)

(6b)

(6)

Formula II

Alternate Preparation of Formula II

Alternatively, the compounds of Formula II can be prepared using an organolithium or organocadmium reagent as illustrated in "Preparation of Aroylbenzoic Acid. Reaction of Aryllithium Reagents with Phthalic Anhydride", *J. Organic Chem.*, 41(7), 1268 (1976), or by Grignard reagent.

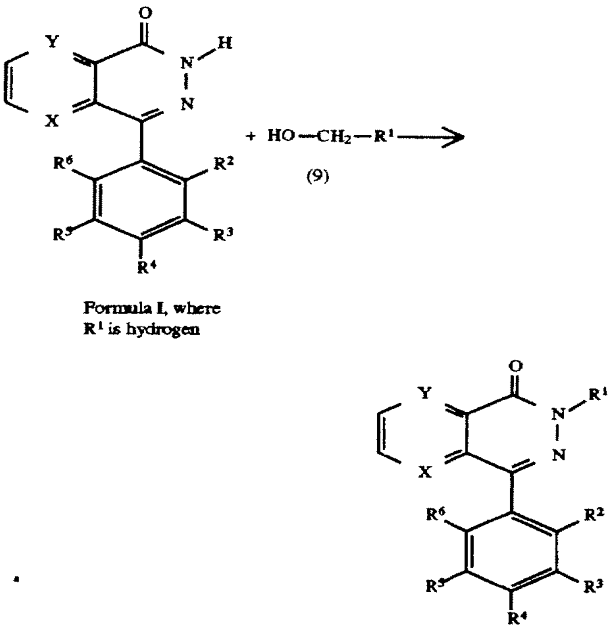
The Grignard preparation is illustrated in Reaction Scheme B (where M is Mg). A Grignard reagent is prepared by adding to a solution of an optionally substituted halogenated aryl compound (Formula 8) in a solvent (e.g., tetrahydrofuran or ethyl ether, preferably tetrahydrofuran) about 1 molar equivalent of magnesium followed by stirring for a period of 12 to 24 hours, preferably 18 hours, at a temperature in the range of about 0° C. to 50° C., preferably room temperature under an inert atmosphere. The Grignard reagent (Formula 8) is added to a benzo or pyridodicarboxylic anhydride (Formula 1) in a solvent (e.g., tetrahydrofuran, methylene chloride, chloroform or ethyl ether, preferably tetrahydrofuran) at a temperature in the range of about 0° C. to −150° C., preferably about −78° C. in a gradual manner over a period of about 2 hours.

After the addition, the solution is stirred for a period of about 15 minutes to 75 minutes, preferably about 45 minutes. An acid (e.g., 1N HCl) is added to the solution at a slight molar excess and stirred for a period of about 1 hour. In the case where Formula 1 is pyridodicarboxylic anhydride (i.e., where either X or Y are nitrogen), the preparation results in two positional isomers of the Formula 6, i.e. Formulae 6a and 6b. Depending on the desired product, i.e., Formula I where X is nitrogen and Y is carbon, or Formula I where X is carbon and Y is nitrogen, the suitable isomer of Formula 6 (Formulae 6a or 6b) is isolated and purified and used in the following preparations as the compound of Formula 6.

Preparation of Formula II

An optionally substituted benzo or pyridobenzoylester (Formula II) can be prepared from a benzo or pyridobenzoylacid (Formula 6) by following the procedures set forth in Reaction Scheme A.

REACTION SCHEME C



(9)

Formula I, where R1 is hydrogen

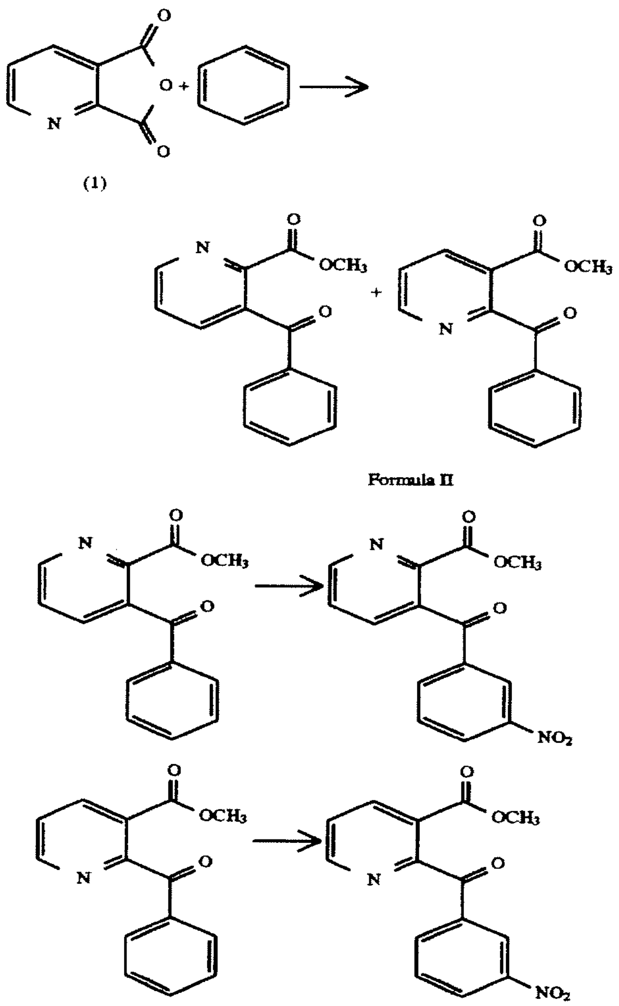
Formula I

Preparation of Formula I Where R^1 is Lower Alkyl, Cycloalkyl, Cycloalkyl Lower Alkyl, Aryl, Aralkyl, Heterocyclo, Heterocyclo Lower-Alkyl, Heteroaryl, or Heteroaralkyl

Alternatively, optionally substituted benzo or pyridopyridazinone compounds (Formula I, where R^1 is lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl) can be prepared from a benzo or pyridopyridazinone compound (Formula I) where R^1 is hydrogen by following Mitsunobu reaction conditions, as illustrated in O. Mitsunobu, *Synthesis*, 1, 1981, and incorporated herein by reference.

To a suspension of a benzo or pyridopyridazinone (Formula I) where R^1 is hydrogen in a solvent (e.g., tetrahydrofuran, diethyl ether, dioxane, preferably tetrahydrofuran) is added about 1 molar equivalent of an optionally substituted alcohol (Formula 9, where R^1 is lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl) and about 1.5 molar equivalent of a trisubstituted phosphine, preferably triphenylphosphine. To the solution is added about 1.5 molar equivalent of a disubstituted azodicarboxylate, such as diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD), preferably diisopropyl azodicarboxylate in a gradual manner. The solution is stirred for a period of about 12 to 24 hours, preferably about 18 hours at a temperature in the range of about 0° C. to 50° C., preferably about 21° C. (room temperature). The solvent is removed and the desired optionally substituted benzo or pyridopyridazinone (Formula I, where R^1 is lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl) is isolated and purified by chromatography.

REACTION SCHEME D



(1)

Formula II

Preparation of Formula II Where X is Carbon and Y is Nitrogen

A pyridophenylketoester (i.e., Formula II where X and Y are carbon or nitrogen, provided at least one is carbon, and R^2, R^3, R^4, R^5 and R^6 are hydrogen) can be prepared following the procedures in U.S. Pat. No. 4,806,535.

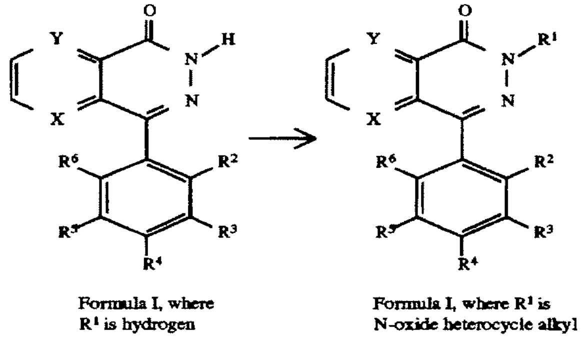
Preparation of Formula II Where X is Carbon, Y is Nitrogen and R^3 is Nitro

A pyrido(3-nitrobenzoyl)ketoester (Formula II where X is carbon, Y is nitrogen and W is nitro) is prepared by dissolving a pyridophenylketoester in an acid (such as hydrochloric acid, nitric acid, sulfuric acid, preferably, sulfuric acid) and adding in a dropwise manner a solution of nitric acid and sulfuric acid (about 1 to 3 molar equivalent of nitric acid with about 2 ml of sulfuric acid per molar equivalent of nitric acid). The solution is stirred for about 10 to 60 minutes, preferably about 30 minutes, cooled and adjusted to a pH of about 7 to 8. The solvent is removed and the desired optionally substituted pyrido-pyridazinone (Formula I, where X is carbon, Y is nitrogen, Z is oxygen, and R^3 is nitro) is isolated and purified by chromatogryaphy.

Preparation of the Salt of Formula I

The pharmaceutically acceptable salts of Formula I are prepared by dissolving a compound of Formula I in a suitable solvent (such as methanol) adding 1 to 3 molar equivalents (preferably about two molar equivalent) of an appropriate acid (such as hydrochloric acid) or base (such as an alkaline earth hydroxide, e.g., lithium hydroxide, calcium hydroxide, potassium hydroxide, sodium hydroxide or the like, preferably sodium hydroxide) with stirring. The salt is isolated by lyophilization or by precipitation, using techniques that will be apparent to those skilled in the art.

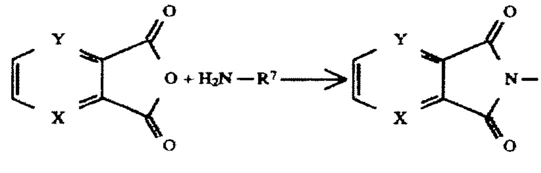
REACTION SCHEME E



Preparation of Formula I Where R^1 is Pyridyl-N-Oxide-Alkyl

Optionally substituted pyridyl-N-oxide-alkyl pyridopyridazinones are prepared by following Mitsunobu reaction conditions, as illustrated in O. Mitsunobu, *Synthesis*, 1, 1981, and incorporated herein by reference. An optionally substituted pyridopyridazinone (Formula I) where R^1 is hydrogen is combined into a suspension with about 1.1 molar equivalents of an optionally substituted pyridylcarbinol N-oxide, about 1.5 molar equivalents of triphenylphosphine in a solvent (e.g., tetrahydrofuran, diethyl ether, dioxane, preferably tetrahydrofuran). The suspension is stirred for a period of about 12 to 24 hours, preferably about 18 hours, at a temperature in the range of 12° to 36° C., preferable about 24° C. The solvent is removed and the desired optionally substituted pyridyl-N-oxide-alkyl pyridopyridazinone is isolated by chromatography, e.g., elution by 100% ethylacetate, followed by 10% methanol/ethylacetate or like solvent systems.

REACTION SCHEME F



(1) (10) (11)

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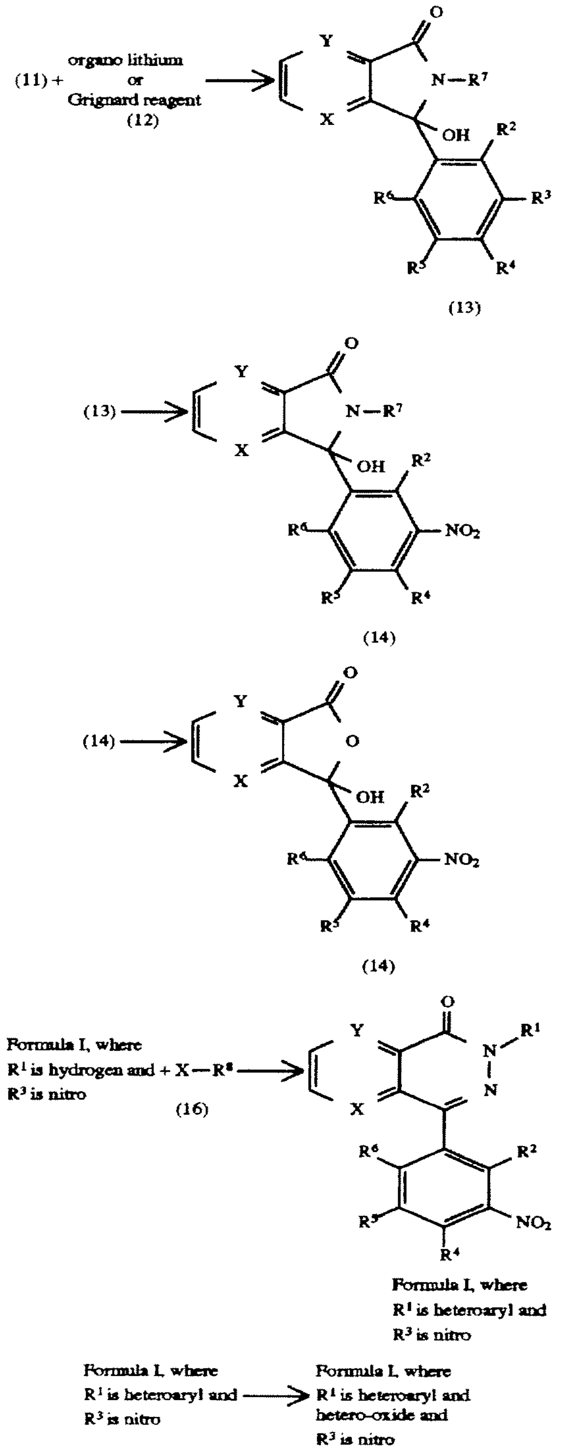
REACTION SCHEME F



Preparation of a Compound of Formula 11

A pyridodicarboxylic anhydride (Formula 1, where X is nitrogen and Y is carbon) is suspended in a solvent (such as toluene, ethyl acetate, tetrahydrofuran, preferably ethyl acetate) and a primary amine (Formula 10, where R^7 is lower alkyl, such as, ethyl, propyl, butyl, hexyl, or n-butyl, preferably n-butyl) is added. The temperature is kept in a range of about −10° C. to about 80° C., preferably room temperature. After a period of about 10 minutes to about 24 hours, preferably about 1 hour, a dehydrating reagent (such as, acetic anhydride, trifluoroacetic anhydride, phosphorous pentoxide, or thionyl chloride, preferably thionyl chloride) is added. The temperature of the mixture is kept in a range of about 0° C. to about 80° C. (depending on the dehydrating reagent) for a period of about 30 minutes to about 4 hours, preferably about 1 hour. The resulting pyridoimide (Formula 11, where X is nitrogen, Y is carbon and R^7 is lower alkyl) is isolated by chromatography or crystallization, preferably crystallization.

Preparation of a Compound of Formula 13

The pyridoimide (Formula 11) is dissolved in a solvent that is inert to Grignard reagents and organolithium reagents (e.g., tetrahydrofuran, diethyl ether, toluene, preferably toluene) and cooled to a temperature in the range of about −100° C. to room temperature, preferably about −65° C. About 0.5 to 3.0 molar equivalent, preferably about 1.0 molar equivalent of an optionally substituted phenyl Grignard reagent or optionally substituted phenyl organolithium reagent, preferably an optionally substituted phenyl Grignard reagent (Formula 8) in either tetrahydrofuran or diethyl ether, preferably tetrahydrofuran is added at such a rate that the internal temperature of the solution is maintained in a range of about −78° C. to about room temperature, preferably about −45° C. After a period of about 10 minutes to about 5 hours, preferably about 30 minutes, the reaction is quenched with dilute hydrochloric acid, dilute acetic acid, aqueous ammonium chloride solution, dilute sulfuric acid, or the like, preferably aqueous ammonium chloride solution. The resulting phenyl pyridolactam compound (Formula 13 where X is nitrogen, Y is carbon, R^7 is lower alkyl, and R^3 is hydrogen) can be isolated by chromatography, crystallization or used as a crude mixture in the next reaction, preferably the product is isolated by crystallization.

Preparation of a Compound of Formula 14 Where R^3 is Nitro

The phenyl pyridolactam compound (Formula 13) is suspended in sulfuric acid and cooled to a temperature in the range of about −10° C. to about 40° C., preferably room temperature. Nitric acid is added at such a rate that the temperature is maintained below about 60° C., preferably below about 45° C. Following the addition of the nitric acid the mixture is stirred for a period of about 10 minutes to about 6 hours, preferably about 30 minutes. The mixture is then added to water and extracted with a water immiscible solvent (e.g., diethyl ether, ethyl acetate, toluene, methylene chloride, preferably ethyl acetate). The resulting optionally substituted nitrophenyl pyridolactam compound (Formula 14 where X is nitrogen, Y is carbon, and R^3 is nitro) can be isolated by chromatography, crystallization or preferably used directly in the next step.

Preparation of a Compound of Formula 15 Where R^3 is Nitro

An optionally substituted nitrophenyl pyridolactam compound (Formula 14) as prepared in the previous step is dissolved or suspended in about 2 to 6 molar equivalent, preferably about 3 molar equivalent, dilute acid (such as, hydrochloric acid, hydrobromic acid, sulfuric acid, preferably hydrochloric acid) and heated to reflux for about 4 hours to about 48 hours, preferably about 24 hours. The optionally substituted nitrophenyl pyridolactone compound (Formula 15 where X is nitrogen, Y is carbon, and R^3 is nitro) is isolated by filtration or neutralization and extraction, followed by crystallization or chromatography, preferably filtration.

Preparation of a Compound of Formula I, Where R^1 is Hydrogen and R^3 is Nitro

An optionally substituted nitrophenyl pyridolactone compound (Formula 15) is mixed with a solvent inert and miscible with hydrazine (e.g., methanol, ethanol, dimethylformamide, preferably methanol) and heated to a temperature in the range of about room temperature to about 80° C., preferably about 65° C. After stirring the reaction for a period of about 1 hour to about 24 hours, preferably about 18 hours, an optionally substituted nitrophenyl pyridopyridazinone (Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is hydrogen, and R^3 is nitro) is isolated by filtration.

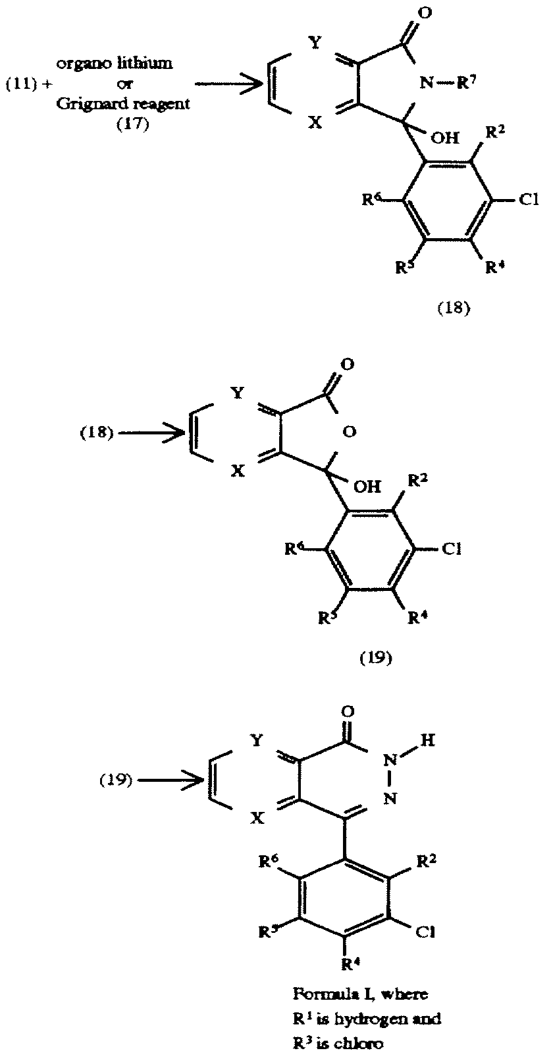
Preparation of a Compound of Formula I Where R^1 is Aryl, Aralkyl, Heterocyclo, Heterocyclo Lower-Alkyl, Heteroaryl, or Heteroaralkyl and R^3 is Nitro

An optionally substituted nitrophenyl pyridopyridazinone (Formula I where X is nitrogen, Y is carbon, R^1 is hydrogen and R^3 is nitro) is dissolved in an aprotic solvent (e.g., tetrahydrofuran, dimethylformamide, dimethylacetamide, preferably dimethylformamide) and treated with a base (e.g., sodium hydride, potassium hydride, preferably sodium hydride). The resulting solution is heated to a temperature in the range of about room temperature to about 95° C., preferably about 65° C. About 1.2 molar equivalent of an optionally substituted aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl alkylating reagent (Formula 16, where R^8 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl) is added. The resulting optionally substituted nitrophenyl heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl pyridopyridazinone (Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^1 is heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl and R^3 is nitro) is isolated by crystallization or chromatography, preferably crystallization.

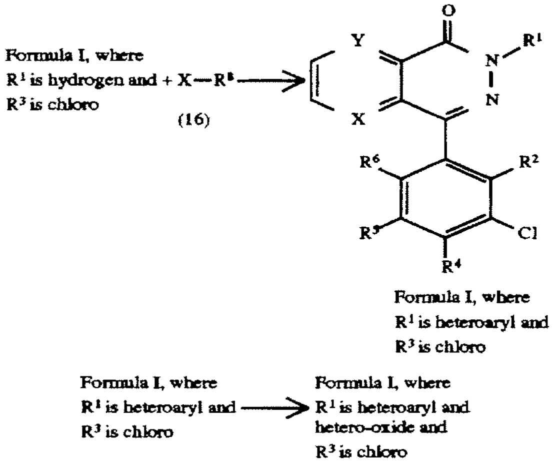
Preparation of a Compound of Formula I Where R^1 is Aryl, Aralkyl, Heterocyclo, Heterocyclo Lower-Alkyl, Heteroaryl, or Heteroaralkyl Hetero-Oxide and R^3 is Nitro

An optionally substituted nitrophenyl aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl pyridopyridazinone (Formula I where X is nitrogen, Y is carbon, R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl and R^3 is nitro) is dissolved in a solvent compatible with peroxyacid (e.g., ethyl acetate, toluene, methylene chloride, acetic acid, preferably methylene chloride) and a peroxyacid (such as, m-chloroperoxybenzoic acid, peroxyacetic acid, trifluoroperoxyacetic acid, magnesium monoperoxy- phthalate, preferably m-chloroperoxybenzoic acid) is added. The resulting mixture is stirred for a period of about 1 hour to about 48 hours, preferably about 18 hours at a temperature in the range of about 0° C. to about 50° C., preferably room temperature. The resulting optionally substituted nitrophenyl heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl hetero-oxide pyridopyridazinone (Formula I, where X is nitrogen, Y is carbon, Z is oxygen, R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl hetero-oxide and R^3 is nitro) is isolated by crystallization or chromatography, preferably crystallization.

REACTION SCHEME F



-continued
REACTION SCHEME F



Preparation of a Compound of Formula 18 Where R^3 is Chloro

An optionally substituted m-chlorophenyl pyridolactam is made by following the procedure for preparing Formula 13 set forth in Reaction Scheme F. An optionally substituted m-chloro Grignard reagent or organolithium reagent (Formula 17) is used in lieu of Formula 12, resulting in the desired optionally substituted m-chlorophenyl pyridolactam (Formula 18, where X is nitrogen, Y is carbon and R^3 is chloro).

Preparation of a Compound of Formula 19 Where R^3 is Chloro

An optionally substituted m-chlorophenyl pyridolactone (Formula 19 where R^3 is chloro) is prepared by following the procedure for preparing Formula 15 set forth in Reaction Scheme F.

Preparation of Compound of Formula I Where R^1 is Hydrogen and R^3 is Chloro

An optionally substituted m-chlorophenyl pyridopyridazinone (Formula I where R^1 is hydrogen and R^3 is chloro) is prepared by following the procedure for preparing Formula I where R^1 is hydrogen and R^3 is nitro set forth in Reaction Scheme F.

Preparation of a Compound of Formula I Where R^1 is Aryl, Aralkyl, Heterocyclo, Heterocyclo Lower-Alkyl, Heteroaryl, or Heteroaralkyl and R^3 is Chloro

An optionally substituted m-chlorophenyl aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl pyridopyridazinone (Formula I where R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl and R^3 is chloro) is prepared by following the procedure for preparing Formula I where R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl and R^3 is nitro set forth in Reaction Scheme F.

Preparation of a Compound of Formula I Where R^1 is Aryl, Aralkyl, Heterocyclo, Heterocyclo Lower-Alkyl, Heteroaryl, or Heteroaralkyl Hetero-Oxide and R^3 is Chloro

An optionally substituted m-chlorophenyl aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl hetero-oxide pyridopyridazinone (Formula I where R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl and R^3 is chloro) is prepared by following the procedure for preparing Formula I where R^1 is aryl, aralkyl, heterocyclo, heterocyclo lower-alkyl, heteroaryl, or heteroaralkyl hetero-oxide and R^3 is nitro set forth in Reaction Scheme F.

PREFERRED COMPOUNDS

Presently preferred are the compound of Formula I where X is nitrogen, Y is carbon, Z is oxygen, R^3 is chloro or nitro and R^1 is ethyl, 2-propyl, 4-pyridyl- methyl, 4-pyridyl-N-oxide-methyl, 3-pyridylmethyl, 3-pyridyl-N-oxide-methyl, benzyl, 3-thienylmethyl or cyclopentylmethyl.

Preferred are the compounds of Formula I where R^3 is chloro and R^1 is ethyl, 4-pyridylmethyl, 4-pyridyl-N-oxide-methyl, 3-pyridylmethyl, 3-pyridyl-N-oxide-methyl, benzyl or 3-thienylmethyl;

particularly the compounds where R^2, R^4, R^5 and R^6 are hydrogen.

Similarly preferred are the compounds of Formula I where R^3 is nitro and R^1 is 4-pyridylmethyl, 4-pyridyl-N-oxide-methyl, 3-pyridylmethyl, 3-pyridyl-N-oxide-methyl, benzyl, 3-thienylmethyl, 2-propyl or cyclopentyl;

particularly the compounds where R^2, R^4, R^5 and R^6 are hydrogen.

Most preferred are the compounds 6-(4-pyridylmethyl)-8-(3- nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one, 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido[2,3-d] pyridazin-5-one, 6-(3-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-(3-pyridyl-N-oxide-methyl)-8-(3- nitrophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-benzyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-ethyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one and 6-(3-thienylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one.

Also most preferred are the compounds 6-(4-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-(3-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-(3-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one, 6-benzyl-8-(3-chloro-phenyl)-pyrido[2,3-d]pyridazin-5-one, 6-(2-propyl)-8-(3-chlorophenyl)-pyrido[2,3-d] pyridazin-5-one, 6-cyclopentyl-8-(3-chlorophenyl)-pyrido [2,3-d]-pyridazin-5-one, and 6-(3-thienylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

PREFERRED PROCESSES AND LAST STEPS

A preferred process for making optionally substituted pyrido[2,3-d]pyridazin-5-one, pyrido-[2,3-d]pyridazin-8-one or benzopyridazin-1-one compounds entails contacting a suitably substituted 2-benzoyl-3-methoxycarbonylpyridine with a suitably substituted hydrazine.

A preferred process for making optionally substituted 2-benzoyl-3-methoxycarbonylpyridine entails contacting a suitably substituted 2-benzoyl-3-carboxypyridine with an esterification reagent.

A preferred process for making optionally substituted 2-benzoyl-3-carboxypyridine entails contacting a suitably substituted benzo or pyridodicarboxylic anhydride with a suitably substituted Grignard reagent.

A preferred process for making 6-substituted pyrido[2,3-d]pyridazin-5-one entails contacting of the 6-unsubsituted pyrido[2,3-d]pyridazin-5-one with a suitable base, followed by the addition of an alkylating agent.

A preferred process for making 7-substituted pyrido[2,3-d]pyridazin-8-one entails contacting of the 7-unsubsituted pyrido[2,3-d]pyridazin-8-one with a suitable base, followed by the addition of an alkylating agent.

A preferred process for making 2-substituted benzopyridazin-1-one entails contacting of the 2-unsubsituted benzopyridazin-1-one with a suitable base, followed by the addition of an alkylating agent.

A preferred process for making 6-substituted pyrido[2,3-d]pyridazin-5-one entails contacting the 6-unsubstituted pyrido[2,3-d]pyridazin-5-one compound with a suitably substituted alcohol and triphenyl phosphine, followed by diisopropyl azodicarboxylate (i.e., Mitsunobu reaction conditions).

A preferred process for making 7-substituted pyrido[2,3-d]pyridazin-8-one entails contacting the 7-unsubstituted pyrido[2,3-d]pyridazin-8-one compound with a suitably substituted alcohol and triphenyl phosphine, followed by diisopropyl azodicarboxylate (i.e., Mitsunobu reaction conditions).

A preferred process for making 2-substituted benzopyridazin-1-one entails contacting the 2-unsubstituted benzopyridazin-1-one compound with a suitably substituted alcohol and triphenyl phosphine, followed by diisopropyl azodicarboxylate (i.e., Mitsunobu reaction conditions).

A preferred process for making 6-substituted pyrido-N-oxide-alkyl[2,3-d]pyridazin-5-one entails suspending the 6-unsubstituted pyrido[2,3-d]pyridazin-5-one compound with a suitably substituted pyridylcarbinol N-oxide and triphenylphosphine, followed by diisopropyl azodicarboxylate (i.e., Mitsunobu reaction conditions).

A preferred process for making 7-substituted pyrido-N-oxide-alkyl[2,3-d]pyridazin-8-one entails suspending the 7-unsubstituted pyrido[2,3-d]pyridazin-8-one compound with a suitably substituted pyridylcarbinol N-oxide and triphenylphosphine, followed by diisopropyl azodicarboxylate (i.e., Mitsunobu reaction conditions).

A preferred process for converting a 6,8-disubstituted pyrido[2,3-d]pyridazin-5-one into the corresponding 6,8-disubstituted pyrido[2,3-d]pyridazin-5-thione entails treating the 6,8-disubstituted pyrido[2,3-d]pyridazin-5-one with a reagent for the thiation of ketones (such as Lawesson's Reagent or phosphorus pentasulfide) in an appropriate solvent, preferably at reflux.

UTILITY, TESTING AND ADMINISTRATION

General Utility

The compounds of this invention, including the pharmaceutically acceptable salts and esters thereof, and the compositions containing them are particularly useful as anti-inflammatory, antasthmatc, immunosuppressive, anti-allograft rejection, anti-graft-vs-host disease, autoimmune disease or analgetic agents. The compounds of this invention act as PDE IV selective inhibitors, thereby modulating cAMP levels. Thus, these compounds are of use for the treatment of conditions or diseases that are modulated by leukocyte cAMP.

For example, inflammation, immunosuppression, autoimmune diseases, graft-vs-host disease and allograft rejection are conditions that are manifested by the proliferation of lymphocytes. The proliferation is triggered by the presence of cAMP at specific levels. Inhibition of lymphocyte proliferation is accomplished by increasing levels of cAMP resulting from the inhibition of lymphocyte phosphodiesterase.

Testing

Potency and selectivity of compounds as inhibitors of PDE IV is determined by following, for example, the procedures described in Example 54, or modifications thereof.

The immunomodulatory and anti-inflammatory activity of the compounds of the invention can be determined by a variety of assays utilizing both in vitro and in vivo procedures.

Inhibition of the proliferation of lymphocytes in response to mitogenic stimulation is determined by the procedures described by Greaves, et al. ["Activation of human T and B lymphocytes by polyclonal mitogens," *Nature,* 248, 698–701 (1974)]., or modifications thereof (see, Example 48).

Inhibition of lymphocyte activation in response to antigenic challenge is determined in vitro by inhibition of a cytolytic T-cell assay (CTL) as describied by Wunderlich, et al., *Nature* (1970), Vol. 228, p. 62, or a modification thereof.

Immune modulation is determined by in vivo procedures utilizing the Jerne Hemolytic Plaque Assay, [Jerne, et al., "The agar plaque technique for recognizing antibody producing cells," *Cell-bound Antibodies*, Amos, B. and Kaprowski, H. editors (Wistar Institute Press, Philadelphia) 1963, p. 109] or a modification thereof (see, Example 47).

Anti-inflammatory activity is determined by the Arachidonic Acid-Induced Mouse Ear Edema Assay [Young, et al., *J. Invest. Derm.,* 82: 367–371 (1984)] (see, Example 49).

Analgetic activity is determined by the phenylquinone-induced Mouse Writhing Assay [Hendershot, et al., *J. Pharmacol. Exp. Ther.,* 125: 237–240 (1959)].

Administration

The compounds of this invention are administered at a therapeutically effective dosage, i.e., that amount which, when administered to a mammal in need thereof, is sufficient to effect treatment, as described above (for example, to reduce or otherwise treat inflammation, pain and/or pyrexia in the mammal). Administration of the active compounds and salts described herein can be via any of the accepted modes of administration for agents that serve similar utilities.

The level of the drug in a formulation can vary within the full range employed by those skilled in the art, e.g., from about 0.01 percent weight (% w) to about 99.99% w of the drug based on the total formulation and about 0.01% w to 99.99% w excipient. Preferably the drug is present at a level of about 10% w to about 70% w.

Generally, an acceptable daily dose is of about 0.0001 to 150 mg per kilogram body weight of the recipient per day, preferably about 0.01 to 75 mg per kilogram body weight per day, and most preferably about 0.1 to 30 mg per kilogram body weight per day. Thus, for administration to a 70 kg person, the dosage range would be about 0.007 mg to 10.5 g per day, preferably about 0.7 to 5.25 g per day, and most preferably about 7.0 mg to 2.1 g per day.

Administration can be via any accepted systemic or local route, for example, via parenteral, oral (particularly for infant formulations), intravenous, nasal, bronchial inhalation (i.e., aerosol formulation) transdermal or topical routes, in the form of solid, semi-solid or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, solutions, suspensions, aerosols, emulsions or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and an active compound of Formula I and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc. Carriers can be selected from the various oils, including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. Other suitable pharmaceutical carriers and their formulations are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

The compounds of this invention are generally administered as a pharmaceutical composition which comprises a pharmaceutical excipient in combination with a compound of Formula I. The level of the drug in a formulation can vary within the full range employed by those skilled in the art, e.g., from about 0.01 percent weight (% w) to about 99.99% w of the drug based on the total formulation and about 0.01% w to 99.99% w excipient. Preferably, the formulation will be about 3.5 to 60% by weight of the pharmaceutically active compound, with the rest being suitable pharmaceutical excipients.

Intravenous Administration

Intravenous injection has proven to be an important route of administration for therapeutic agents. The compounds of the present invention can be administered via this route, for example, by dissolving the compound, salt, ester or ether in a suitable solvent (such as water or saline) or incorporation in a liposomal formulation followed, by dispersal into an acceptable infusion fluid. A typical daily dose of a compound of the invention can be administered by one infusion, or by a series of infusions spaced over periodic intervals.

Oral Administration

Oral administration can be used to deliver the compound of Formula I using a convenient daily dosage regimen which can be adjusted according to the degree of affliction or for renal impairment, or to compensate for the toxic effects of other medications administered contemporaneously. For such oral administration, a pharmaceutically acceptable, non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain between 0.01 wt/wt % and 99.99 wt/wt % of the compound of Formula I, but preferably such compositions will contain between 25 wt/wt % and about 80 wt/wt %.

Preferably the compositions will take the form of a capsule, pill or tablet and thus the composition will contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant such as starch or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as a starch, polyvinylpyrrolidone, gum acacia, gelatin, cellulose and derivatives thereof, and the like. For oral administration to infants, a liquid formulation (such as a syrup or suspension) is preferred.

Aerosol Administration

Aerosol administration is an effective means for delivering a therapeutic agent directly to the respiratory tract. Some of the advantages of this method are: 1) it circumvents the effects of enzymatic degradation, poor absorption from the gastrointestinal tract, or loss of the therapeutic agent to the hepatic first-pass effect; 2) it administers therapeutic agents which would otherwise fail to reach their target sites in the respiratory tract due to their molecular size, charge or affinity to extra-pulmonary sites; 3) it provides for fast absorption into the body via the aveoli of the lungs; and 4) it avoids exposing other organ systems to the therapeutic agent, which is important where exposure might cause undesirable side effects. For these reasons, aerosol administration is particularly advantageous for treatment of asthma, local infections of the lung, and other diseases or disease conditions of the lung and respiratory tract.

There are three types of pharmaceutical inhalation devices, nebulizers inhalers, metered-dose inhalers (MDI) and dry powder inhalers (DPI). Nebulizer devices produce a stream of high velocity air that causes the therapeutic agent (which has been formulated in a liquid form) to spray as a mist which is carried into the patient's respiratory tract. MDIs typically have the formulation packaged with a compressed gas. Upon actuation, the device discharges a measure amount of therapeutic agent by compressed gas, thus affording a reliable method of administering a set amount of agent. Historically, MDIs have used chlorofluorocarbons (CFC) as the compressed gas to propel the therapeutic agent. In recent years, CFCs have been linked with the depletion of the earth's ozone layer. As a result of this, alternative propellants that are non-ozone threatening are being sought out as potential replacements for CFCs.

DPIs administer therapeutic agents in the form of a free flowing powder that can be dispersed in the patient's inspiratory air-stream during breathing by the device. In order to achieve a free flowing powder, the therapeutic agent is formulated with an excipient, such as lactose. A measured amount of the therapeutic is stored in a capsule form and is dispensed to with each actuation. Examples of DPIs being used are Spinhaler® (for the administration of disodium cromoglycate), Rotahaler® (for albuterol) and Turbuhaler® (for terbutaline sulfate). All of the above methods can be used for administering the present invention, particularly for the treatment of asthma and other similar or related respiratory tract disorders.

Liposomal Formulations

Pharmaceutical formulations based on liposomes have recently reached human clinical trials. Their benefits are believed related to favorable changes in tissue distribution and pharmacokinetic parameters that result from liposome entrapment of drugs, and may be applied to the compounds of the present invention by those skilled in the art.

The formulations can be designed to either target drug to disease sites [see: Lopez-Berestein et al., *J. Infect. Dis.*, 151: 704–710 (1985); Gotfredsen et al., *Biochemical Pharmacology*, 32: 3389–3396 (1983)]; or to the reticuloendothelial system [see Eppstein et al., *Int. J. Immunotherapy*, 2: 115–126 (1986)], to increase duration of drug action [see: Gabizon et al., *Cancer Res.*, 42: 4734 (1982); Eppstein et al., *Delivery Systems for Peptide Drugs*, Eds. S. S. Davis, L. Illum and E. Tomlinson, Plenum Pub. Corp., New York, pp. 277–283; C. A. Hunt, *Biochemica et Biophysica Acta.*, 719: 450–463 (1982); and Senior et al., *Biochemica et Biophysica Acta.*, 839: 1–8 (1985)], or to divert a drug away from organs that are particularly sensitive to its toxic effects [see: Weinstein et al., *Pharmac. Ther.*, 24: 207–233 (1983); Olson et al., *Eur. J. Cancer Clin. Oncol.*, 18: 167–176 (1982); and Gabzion et al., supra.].

Controlled release liposomal liquid pharmaceutical formulations for injection or oral administration are described in U.S. Pat. No. 4,016,100. Liposomal applications for oral drug delivery of a lyophilized liposome/peptide drug mixture filled into intestine capsules have also been suggested, see U.S. Pat. No. 4,348,384. The foregoing are incorporated herein by reference.

Suppositories

For systemic administration via suppository, traditional binders and carriers include, for example, polyalkaline glycol or triglycerides [e.g., PEG 1000 (96%) and PEG 4000 (4%)]. Such suppositories may be formed from mixtures containing active ingredients in the range of from about 0.5 wt/wt % to about 10 wt/wt %; preferably from about 1 wt/wt % to about 2 wt/wt %.

Liquids

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound (about 0.5% to about 20%), as described above, and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa., 16th Ed., 1980. The composition to be administered will, in any event, contain a quantity of the active compound(s) in a pharmaceutically effective amount for relief of the particular condition being treated in accordance with the teachings of this invention.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Example 1

Preparation of 7-(3-nitrophenyl)-furo-[3,4-b] pyridin-5-one

1A. Formula 4, Where X is nitrogen, Y is carbon, R^3 is nitro, and R^2, R^4, R^5 and R^6 are hydrogen

A solution of 2,3-pyridinyldicarboxylic anhydride (20 g, 134 mmoles) in 25 ml of anhydrous methanol was refluxed for 30 minutes. The methanol was removed and the two positional isomers (2-carboxy-3-methoxycarbonylpyridine and 2-methoxycarbonyl-3-carboxypyridine) are isolated and separated. The 2-carboxy-3-methoxycarbonyl pyridine was added to a solution of 3-nitrobenzaldehyde (101.35 g, 670 mmoles) in p-cymene (105 ml, 670 mmoles) and heated at 180° C. for 13.5 hours. The solution was cooled, filtered and the excess 3-nitrobenzaldehyde and p-cymene removed by bulb to bulb distillation using a Kugelrohr apparatus. The residue was triturated with methylene chloride and the solid residue was discarded. The product was isolated from the filtrate yielding 12.7 g of 7-(3-nitrophenyl)-furo[3,4-b] pyridin-5-one (37.2%).

1B. Preparation of Other Compounds of Formula 4, Where X is nitrogen and Y is carbon

By following the procedures of Example 1A and substituting for 3-nitrobenzaldehyde with the following:

3-methoxybenzaldehyde;
4-methoxybenzaldehyde;
4-methylbenzaldehyde;
3-bromobenzaldehyde;
3-chlorobenzaldehyde;
3-nitro-4-chlorobenzaldehyde;
3-nitro-4-methylbenzaldehyde;
3-methoxycarbonylbenzaldehyde;
4-methoxycarbonylbenzaldehyde;
3-methoxycarbonyl-4-methyl-benzaldehyde;
4-carboxybenzaldehyde;
4-carbamoylbenzaldehyde;
4-N,N-dimethylcarbamoylbenzaldehyde; and
3,4-methylenedioxybenzaldehyde;

there are obtained the following respective compounds:

7-(3-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-bromophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-chlorophenyl)-furo[3,4-b]pyridin -5-one;
7-(3-nitro-4-chlorophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl-4-methyl)-furo-[3,4-b] pyridin-5-one;
7-(4-carboxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-carbamoylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-N,N-dimethylcarbamoylphenyl)-furo-[3,4-b]pyridin-5-one; and
7-(3,4-methylenedioxyphenyl)-furo[3,4-b]pyridin-5-one.

Example 2

Preparation of 2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine

2A. Formula 5, Where X is nitrogen, Y is carbon, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

7-(3-nitrophenyl)-furo[3,4-b]pyridin-5-one was suspended in methanol (100 ml), solid potassium hydroxide (4.5 gm, 80 mmoles) was added and the mixture refluxed for 6 hours under an inert atmosphere yielding 2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine.

2B. Preparation of Other Compounds of Formula 5 Where X is nitrogen and Y is carbon

By following the procedures of Example 2A and substituting for 7-(3-nitrophenyl)-furo[3,4-b]pyridin-5-one with the following:

7-(3-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-bromophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-chlorophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-chlorophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonyl-4-methylphenyl)-furo-[3,4-b]-pyridin-5-one;
7-(4-carboxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-carbamoylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-N,N-dimethylcarbamoylphenyl)-furo-[3,4-b]-pyridin-5-one; and
7-(3,4-methylenedioxyphenyl)-furo[3,4-b]pyridin-5-one;

there are obtained the following compounds:

2-(hydroxymethyl-3-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-bromophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carboxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carbamoylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-N,N-dimethylcarbamoyl-phenyl)-3-carboxypyridine; and
2-(hydroxymethyl-3,4-methylenedioxyphenyl)-3-carboxypyridine.

Example 3

Preparation of 2-(3-nitrobenzoyl)-3-carboxypyridine

3A. Formula 6, Where X is nitrogen, Y is carbon, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

To a solution of 2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine (2.25 g, 8.2 mmoles) in methylene chloride (20 ml), pyridinium dichromate (4.63 g, 12.3 mmoles) was added and stirred at room temperature (22° C.) for 18 hours. The solution was filtered and the solvent removed yielding 3.43 g of crude 2-(3-nitro-benzoyl)-3-carboxypyridine.

3B. Preparation of Other Compounds of Formula 6 Where X is nitrogen and Y is carbon

By following the procedures of Example 3A and substituting for 2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine with the following:

2-(hydroxymethyl-3-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-bromophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonyl-4-methylphenyl)-carboxypyridine;
2-(hydroxymethyl-4-carboxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carbamoylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-N,N-dimethylcarbamoyl-phenyl)-3-carboxypyridine; and
2-(hydroxymethyl-3,4-methylenedioxyphenyl)-3-carboxypyridine;

there are obtained the following compounds:

2-(3-methoxybenzoyl)-3-carboxypyridine;
2-(4-methoxybenzoyl)-3-carboxypyridine;
2-(4-methylbenzoyl)-3-carboxypyridine;
2-(3-bromobenzoyl)-3-carboxypyridine;
2-(3-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-methylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(4-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-carboxypyridine;
2-(4-carboxybenzoyl)-3-carboxypyridine;
2-(4-carbamoylbenzoyl)-3-carboxypyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-carboxypyridine; and
2-(3,4-methylenedioxybenzoyl)-3-carboxypyridine.

Example 4

Preparation of 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine

4A. Formula II, Where X is nitrogen, Y is carbon, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

2-(3-Nitrobenzoyl)-3-carboxypyridine was dissolved in 20 ml of methylene chloride and the solution was cooled in an ice/water bath. Diazomethane (25 ml, 0.33M) was added to the solution and stirred for 2 hours. The solvent was removed and the residue chromatographed (methylene chloride) yielding 0.26 g of 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine.

4B. Preparation of Other Compounds of Formula II Where X is nitrogen and Y is carbon

By following the procedures of Example 4A and substituting for 2-(3-Nitrobenzoyl)-3-carboxypyridine with the following:

2-(3-methoxybenzoyl)-3-carboxypyridine;
2-(4-methoxybenzoyl)-3-carboxypyridine;
2-(4-methylbenzoyl)-3-carboxypyridine;
2-(3-bromobenzoyl)-3-carboxypyridine;
2-(3-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-methylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(4-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-carboxypyridine;
2-(4-carboxybenzoyl)-3-carboxypyridine;
2-(4-carbamoylbenzoyl)-3-carboxypyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-carboxypyridine; and
2-(3,4-methylenedioxybenzoyl)-3-carboxypyridine;

there are obtained the following compounds:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxybenzoyl)-3-methoxy-carbonylpyridine;
2-(4-methylbenzoyl)-3-methoxy-carbonylpyridine;
2-(3-bromobenzoyl)-3-methoxy-carbonylpyridine;
2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and
2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine.

Example 5

Preparation of 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one

5A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^3 is nitro and R^1, R^2, R^4, R^5 and R^6 are hydrogen

To a solution of 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine (0.58 g, 2 mmoles) dissolved in ethanol (50 ml), was added hydrazine monohydrate (0.25 ml, 5.1 mmoles). The mixture was refluxed for 4 hours, cooled and ethyl acetate (50 ml) was added. The solid material was collected and dried, yielding 0.58 g of 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one, mp 302° C.

5B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 5A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;
2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and

2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]-pyridazin-one;

8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-one;

8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;

8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;

8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;

8-(4-N,N-dimethyl carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and

8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 6

Preparation of 6-phenyl-8-(3-nitrophenyl)pyrido [2,3-d]pyridazin-5-one

6A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is phenyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

To a solution of 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine (0.26 g, 0.97 mmoles) dissolved in ethanol (50 ml), was added phenylhydrazine (0.25 ml, 2.4 mmoles). The mixture was refluxed for 168 hours. The solvent was removed and the solid was precipitated from ethanol and dried, yielding 0.07 g of 6-phenyl-8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one (21%), mp 167° C.

6B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 6A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;

2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;

2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;

2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine;

2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;

2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;

2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and

2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

6-phenyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-phenyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-phenyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-one;

6-phenyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-one;

6-phenyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-one;

6-phenyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;

6-phenyl-8-(3-nitro-1-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;

6-phenyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;

6-phenyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;

6-phenyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-phenyl-8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-phenyl-8-(4-carbamoylphenyl)-pyrido-[2,3-d]-pyridazin-5-one;

6-phenyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and

6-phenyl-8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 7

Preparation of 6-benzyl-8-(3-nitrophenyl)pyrido-[2,3-d]pyridazin-5-one

7A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

To a solution of 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine (0.20 g, 0.75 mmoles) dissolved in ethanol (50 ml), was added benzylhydrazine hydrochloride (0.36 g, 1.86 mmoles). The mixture was heated to 85° C. for 144 hours. The mixture was purified by chromatography yielding 0.11 g of 6-benzyl-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one (41%), mp 132° C.

7B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 7A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;

2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;

2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;

2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine;

2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;

2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(4- carboxybenzoyl)-3-methoxycarbonylpyridine;

2-(4- carbamoylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and

2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

6-benzyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-benzyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

6-benzyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-one;
6-benzyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-one;
6-benzyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-one;
6-benzyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one; 6-benzyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4- carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-carbamoylphenyl)-pyrido-[2,3-d]-pyridazin-5-one;
6-benzyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-benzyl-8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 8

Preparation of 6-ethyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one

8A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is ethyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

8-(3-Nitrophenyl)-pyrido[2,3-d]pyridazin-5-one (0.20 g, 0.75 mmoles), sodium hydride (0.04 g, 0.9 mmoles) and iodoethane (0.3 ml, 3.75 mmoles) was dissolved in 25 ml of tetrahydrofuran and refluxed under a nitrogen atmosphere for 18 hours. The solvent was removed and the product chromatographed and eluted with 100% methylene chloride yielding 0.05 g of 6-ethyl-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one (22.6%), mp. 138° C.

8B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 8A and substituting for 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4- carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-5-one;

there are obtained the following compounds:

6-ethyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-one;
6-ethyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-one;
6-ethyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-ethyl-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 9

Preparation of 6-(4-pyridylmethyl)-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one

9A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridylmethyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen

To a solution of 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one (0.16 g, 0.6 moles) in tetrahydrofuran (50 ml) was added potassium carbonate (0.19 g, 7.1 moles), potassium iodide (1.0 g, 6.0 moles) and 4-picolyl chloride (0.98 g, 6.0 mmoles). The mixture was stirred and refluxed under an inert atmosphere for 18 hours. The solvent was removed under vacuum. The residue was partitioned in methanol/ethyl acetate (1:1), chromatographed in 100% hexane followed by 100% ethyl acetate. The remaining solvent was removed yielding 0.58 g of crude 6-(4-pyridylmethyl)-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one, which was recrystallized from methanol yielding 0.08 g of the pure compound (37.1%), mp. 167°–168° C.

9B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 9A and substituting for 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-5-one;

there are obtained the following compounds:

6-(4-pyridylmethyl)-8-(3-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;

6-(4-pyridylmethyl)-8-(3-bromophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-carboxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-carbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one; and
6-(4-pyridylmethyl)-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 10

Preparation of 7-(3-chlorophenyl)-furo[3,4-b]pyridin-5-one

10A. Formula 4, Where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

A solution of 2,3-pyridinyldicarboxylic anhydride (20.0 g, 134 mmoles) in methanol (25 g, 617 mmoles) was refluxed for 10 minutes and the solvent removed. The solution was filtered and the remaining solvent was removed. The residue was added to 3-chlorobenzaldehyde (76 ml, 671 moles) and p-cymene (105 ml, 671 moles) and the mixture was stirred at 180° C. for 18 hours under an inert atmosphere. The solvent was removed and the residue was chromatographed yielding 7-(3-chlorophenyl)-furo[3,4-b]pyridin-5-one.

10B. Preparation of Other Compounds of Formula 4, Where X is nitrogen and Y is carbon

By following the procedures of Example 10A and substituting 3-chlorobenzaldehyde with the following:
3-methoxybenzaldehyde;
4-methoxybenzaldehyde;
4-methylbenzaldehyde;
3-bromobenzaldehyde;
3-nitrobenzaldehyde;
3-nitro-4-chlorobenzaldehyde;
3-nitro-4-methylbenzaldehyde;
3-methoxycarbonylbenzaldehyde;
4-methoxycarbonylbenzaldehyde;
3-methoxycarbonyl-4-methyl-benzaldehyde;
4-carboxybenzaldehyde;
4-carbamoylbenzaldehyde;
4-N,N-dimethylcarbamoylbenzaldehyde; and
3,4-methylenedioxybenzaldehyde;
there are obtained the following respective compounds:
7-(3-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-bromophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitrophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-chlorophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;

7-(4-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl-4-methyl)-furo[3,4-b]pyridin-5-one;
7-(4-carboxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-carbamoylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-N,N-dimethylcarbamoylphenyl)-furo-[3,4-b]pyridin-5-one; and
7-(3,4-methylenedioxyphenyl)-furo[3,4-b]pyridin-5-one.

Example 11

Preparation of 2-(hydroxymethyl-3-chlorophenyl)-3-carboxypyridine

11A. Formula 5, Where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

7-(3-Chlorobenzoyl)-furo[3,4-b]pyridin-5-one (4.77 g, 19.4 mmoles) and 0.2N sodium hydroxide (21.4 mmoles) was dissolved in methanol and tetrahydrofuran (100 ml, 1:1). The mixture was heated to 75° C. and stirred for 3 hours and cooled. 1N Hydrochloric acid (20 ml) and water (200 ml) was added to the mixture. The resulting solution was extracted with ethyl acetate and dried over Na_2SO_4. The solution was filtered and the solvent removed yielding 4.94 g (96.8%) of 2-(hydroxymethyl-3-chlorophenyl)-3-carboxypyridine.

11B. Preparation of Other Compounds of Formula 5, Where X is nitrogen, Y is carbon

By following the procedures of Example 11A and substituting for 7-(3-chlorophenyl)-furo[3,4-b]pyridin-5-one with the following:
7-(3-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methoxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-bromophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitrophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-chlorophenyl)-furo[3,4-b]pyridin-5-one;
7-(3-nitro-4-methylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonylphenyl)-furo[3,4-b]pyridin-5-one;
7-(3-methoxycarbonyl-4-methylphenyl)-furo[3,4-b]-pyridin-5-one;
7-(4-carboxyphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-carbamoylphenyl)-furo[3,4-b]pyridin-5-one;
7-(4-N,N-dimethylcarbamoylphenyl)-furo[3,4-b]-pyridin-5-one; and
7-(3,4-methylenedioxyphenyl)-furo[3,4-b]pyridin-5-one;
there are obtained the following compounds:
2-(hydroxymethyl-3-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-bromophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-carboxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-carboxy-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carboxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carbamoylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-N,N-dimethylcarbamoylphenyl)-3-carboxypyridine; and
2-(hydroxymethyl-3,4-methylenedioxyphenyl)-3-carboxypyridine.

Example 12

Preparation of 2-(3-chlorobenzoyl)-3-carboxypyridine

12A. Formula 6, Where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

2-(Hydroxymethyl-3-chlorophenyl)-3-carboxypyridine was dissolved in a solution of methylene chloride/glacial acetic acid (5:1). Pyridinium dichromate (10.95 g, 29 mmoles) was added and the solution was stirred at room temperature under an inert atmosphere. The solvent was removed yielding 2-(3-chlorobenzoyl)-3-carboxypyridine.

12B. Preparation of Other Compounds of Formula 6 Where X is nitrogen and Y is carbon

By following the procedures of Example 12A and substituting for 2-(hydroxymethyl-3-chlorophenyl)-3-carboxypyridine with the following:

2-(hydroxymethyl-3-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-bromophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitrophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-chlorophenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-nitro-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-methoxycarbonylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-3-methoxycarbonyl-4-methylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carboxyphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-carbamoylphenyl)-3-carboxypyridine;
2-(hydroxymethyl-4-N,N-dimethyl-carbamoylphenyl)-3-carboxypyridine; and
2-(hydroxymethyl-3,4-methylenedioxyphenyl)-3-carboxypyridine;

there are obtained the following compounds:

2-(3-methoxybenzoyl)-3-carboxypyridine;
2-(4-methoxybenzoyl)-3-carboxypyridine;
2-(4-methylbenzoyl)-3-carboxypyridine;
2-(3-bromobenzoyl)-3-carboxypyridine;
2-(3-nitrobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-methylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(4-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-carboxypyridine;
2-(4-carboxybenzoyl)-3-carboxypyridine;
2-(4-carbamoylbenzoyl)-3-carboxypyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-carboxypyridine; and
2-(3,4-methylenedioxybenzoyl)-3-carboxypyridine.

Example 13

Preparation of 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine

13A. Formula II, Where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

2-(3-Chlorobenzoyl)-3-carboxypyridine was dissolved in a solution of 10% methanol and methylene chloride. Diazomethane was added and the solution was stirred for 1 hour at room temperature. The solution was extracted with methylene chloride followed by methylene chloride +1% methanol The desired product was combined from recrystallization (ethyl ether/hexane) and from the filtrate to yield 1.23 g of 2-(3-chlorobenzoyl)-3-methoxycarbonyl-pyridine.

13B. Preparation of Other Compounds of Formula II Where X is nitrogen and Y is carbon

By following the procedures of Example 13A and substituting for 2-(3-chlorobenzoyl)-3-carboxypyridine with the following:

2-(3-methoxybenzoyl)-3-carboxypyridine;
2-(4-methoxybenzoyl)-3-carboxypyridine;
2-(4-methylbenzoyl)-3-carboxypyridine;
2-(3-bromobenzoyl)-3-carboxypyridine;
2-(3-nitrobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-chlorobenzoyl)-3-carboxypyridine;
2-(3-nitro-4-methylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(4-methoxycarbonylbenzoyl)-3-carboxypyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-carboxypyridine;
2-(4-carboxybenzoyl)-3-carboxypyridine;
2-(4-carbamoylbenzoyl)-3-carboxypyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-carboxypyridine; and
2-(3,4-methylenedioxybenzoyl)-3-carboxypyridine;

there are obtained the following compounds:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and
2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine.

Example 14

Preparation of 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one

14A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is hydrogen, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

A solution of 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine (0.55 g, 2.0 mmoles), hydrazine hydrate (0.15 ml, 3.0 mmoles) in ethanol (50 ml) was stirred at 100° C. for 18 hours under an inert atmosphere. The solution was cooled and solid in the solution was collected, washed with ethyl ether and dried to give 0.26 g of 8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin-5-one (50.5%), mp 268°–269° C.

14B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 14A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;

2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and
2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 15

Preparation of 6-phenyl-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one

15A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is phenyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

A solution of 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine (0.30 g, 1.09 mmoles), phenylhydrazine (0.27 ml, 2.72 mmoles) in ethanol (18 ml) was stirred at 100° C. for 72 hours. The solution was cooled and the solvent was removed. The residue was triturated with methanol to give a white solid, which was collected and air dried, yielding 0.26 g of 6-phenyl-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (71.5%), mp 144°–145° C.

15B. Preparation of Other Compounds of Formula I Where X nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 15A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
(4-methoxybenzoyl)-3-methoxycarbonylpyridine;
(4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and
2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

6-phenyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-one;
6-phenyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-one;
6-phenyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-one;
6-phenyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-carbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-phenyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-phenyl-8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 16

Preparation of 6-benzyl-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one

16A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is benzyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen

A solution of 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine (0.30 g, 1.09 mmoles), benzylhydrazine hydrochloride (0.53 ml, 2.72 mmoles) in ethanol (18 ml) was refluxed for 132 hours (5 days). The solution was cooled and the solvent was removed. The residue was dissolved in ethanol (minimal amount used) and stored in a freezer to induce crystallization. The solid material was collected from the solution, chromatographed, eluted with 100% methylene chloride, and crystallized from methanol to yield 0.21 g of 6-benzyl-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one (55.4%), mp 141° C.

16B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen

By following the procedures of Example 16A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with the following:

2-(3-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methoxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-bromobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-chlorobenzoyl)-3-methoxycarbonylpyridine;
2-(3-nitro-4-methylbenzoyl)-3-methoxy-carbonylpyridine;
2-(3-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;

2-(4-methoxycarbonylbenzoyl)-3-methoxycarbonylpyridine;
2-(3-methoxycarbonyl-4-methylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-carboxybenzoyl)-3-methoxycarbonylpyridine;
2-(4-carbamoylbenzoyl)-3-methoxycarbonylpyridine;
2-(4-N,N-dimethylcarbamoylbenzoyl)-3-methoxycarbonylpyridine; and
2-(3,4-methylenedioxybenzoyl)-3-methoxycarbonylpyridine;

there are obtained the following compounds:

6-benzyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-benzyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-benzyl-8-(4-carbamoylphenyl)-pyrido-[2,3-d]-pyridazin-5-one;
6-benzyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-benzyl-8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 17

Preparation of 6-ethyl-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one

17A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is ethyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

To a suspension of 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (0.32 g, 1.24 mmoles) in tetrahydrofuran (50 ml) was added sodium hydride (0.06 g, 1.4 mmoles). The mixture was stirred for 1 hour at room temperature. Iodoethane (0.48 g, 3.1 mmoles) was added and the solution was refluxed for 18 hours under an inert atmosphere. The mixture was cooled and acidified by the addition of 50 ml of 1N HCl. The desired compound was isolated by extraction with ethyl acetate (extracted until no product detected in aqueous layer), washed with sodium bicarbonate, brine and dried over magnesium sulfate. The solution was filtered and the solvent removed yielding 0.85 g of the crude desired product. The product was purified by recrystallization from methanol to yield 0.24 g of 6-ethyl-8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin-5-one (67.8%), mp. 117°–118° C.

17B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen.

By following the procedures of Example 17A and substituting for 8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonylphenyl)-pyrido [2,3-d]pyridazin-5-one;
8-(4-methoxycarbonylphenyl)-pyrido [2,3-d]pyridazin-5-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]-pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-5-one;

there are obtained the following compounds:

6-ethyl-8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-ethyl-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-one;
6-ethyl-8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
6-ethyl-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one; and
6-ethyl-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 18

Preparation of 6-(2-propyl)-8-(3-chlorophenyl)pyrido [2,3-d]pyridazin-5-one

18A. Formula i, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 2-propyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

To a suspension of 8-(3-chlorophenyl)pyrido [2,3-d]pyridazin-5-one (0.33 g, 1.28 mmoles) in tetrahydrofuran (50 ml) was added sodium hydride (0.07 g, 1.53 mmoles). The mixture was stirred at room temperature for 30 minutes under an inert atmosphere. 2-Iodopropane (0.64 ml, 6.4 mmoles) was added and the mixture refluxed for 48 hours. The mixture was cooled and acidified by the addition of 50 ml of 1N HCl. The mixture was extracted with ethyl acetate (3×50 ml) and dried over magnesium sulfate. The solution was filtered and the solvent removed to yield the crude product. The product was purified by crystallization (ethyl ether/hexane) yielding 0.15 g of 6-(2-propyl)-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (39%), mp 80°–81° C.

18B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen.

By following the procedures of Example 18A and substituting for 8-(3-chlorophenyl) -pyrido[2,3-d]pyridazin-5-one with the following:

8-(3-methoxyphenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3- d]pyridazin -5-one;
8-(3-bromophenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazino-5-one;
8-(3- nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3- d]pyridazin-5- one;
8-(4- carbamoylphenyl)-pyrido[2,3- d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

there are obtained the following compounds:

6-(2-propyl)-8-(3-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-methylphenyl)-pyrido-[2,3-d]-pyridazin-5-one;
6-(2-propyl)-8-(3-bromophenyl)-pyrido-[2,3-d]-pyridazin-5- one;
6-(2-propyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]-pyridazin-5-one;
6-(2-propyl)-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-carboxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-carbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-propyl)-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-(2-propyl)-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 19

Preparation of 6-(4-pyridylmethyl)-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one

19A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridylmethyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

To a suspension of 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (0.50 g, 1.90 mmoles) in dimethylformamide (50 ml) was added potassium carbonate (3.22 g, 23.3 mmoles). The mixture was stirred at room temperature for 1 hour under an inert atmosphere. 4-Picolyl chloride (1.59 g, 9.7 mmoles) and potassium iodide (1.61 g, 9.7 mmoles) was added, the mixture was stirred at 80° C. for 168 hours (7 days). The mixture was cooled, the insoluble material was filtered out and the solvent removed yielding 2.13 g of the crude product. The product was purified by chromatography, eluted with 100% ethylacetate and crystallized from methanol yielding 0.091 g of 6-(4-pyridylmethyl)-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (13.7%), mp 152° C.

19B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen.

By following the procedures of Example 19A and substituting for 8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)- pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)- pyrido[2,3-d]pyridazin-5-one;
8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4- carboxyphenyl)- pyrido[2,3-d]pyridazin-5-one;
8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one;

there are obtained the following compounds:

6-(4-pyridylmethyl)-8-(3-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methoxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-bromophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6(4-pyridylmethyl)-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido- [2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-carboxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-carbamoylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridylmethyl)-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido -[2,3-d]pyridazin-5-one; and
6-(4-pyridylmethyl)-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 20

Preparation of 2-(3-chlorobenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-chlorobenzoyl) pyridine Using A Grignard Reagent

20A. Formula 6, Where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

A Grignard reagent was prepared by adding 1-bromo-3-chlorobenzene (11.75 ml, 100 mmoles) to a suspension of magnesium (2.43 g, 100 mmoles) in tetrahydrofuran (100 ml) and stirred at room temperature for 18 hours under an inert atmosphere. The Grignard reagent was added in a dropwise manner to a solution of 2,3-pyridyldicarboxylic anhydride (14.91ml, 100mmoles) in tetrahydrofuran (200 ml) at a temperature of −78° C. over a 2 hour period. After the addition the solution was stirred for an additional 45 minutes. 1N HCl (110 ml) was added and the solution was stirred for 1 hour. The desired product was extracted with ethyl acetate, washed in brine and dried over Na_2SO_4 yielding 2-(3-chlorobenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-chlorobenzoyl)pyridine.

2-(3-Chlorobenzoyl)-3-carboxypyridine (Formula 6, where X is nitrogen, Y is carbon, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen) can be converted to the corresponding compounds of Formulae II and I by following the procedures of Examples 13–19, respectively.

20B. Preparation of Other Compounds of Formula II Where X is nitrogen and Y is carbon.

By following the procedures of Example 20A and substituting for 1-bromo-3-chlorobenzene with the following:

1-bromo-3-thiomethylbenzene;
1-bromo-3-methylbenzene;
1-bromo-3-trifluoromethylbenzene;
1-bromo-3,4-methylenedioxybenzene;
1-chloro-3-thiomethylbenzene;
1-chloro-3-methylbenzene;
1-chloro-3-trifluoromethylbenzene;
1-chloro-3,4-methylenedioxybenzene;
1-chloro-3-methoxybenzene;
1-chlorobenzene; and
1-bromobenzene;

there are obtained the following respective compounds:

2-(3-thiomethylbenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-thiomethylbenzoyl)pyridine;
2-(3-methylbenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-methylbenzoyl)pyridine;
2-(3- trifluoromethylbenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-trifluoromethylbenzoyl)pyridine;
2-(3,4 -methylenedioxybenzoyl)-3-carboxypyridine and 2-carboxy-3-(3,4-methylenedioxybenzoyl)pyridine;
2-(3-thiomethylbenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-thiomethylbenzoyl)pyridine;
2-(3-methylbenzoyl)-3-carboxypyridine and 2-carboxy-3 -(3-methylbenzoyl)pyridine;
2-(3- trifluoromethylbenzoyl)-3-carboxypyridine and 2-carboxy-3-(3- trifluoromethylbenzoyl)pyridine;
2-(3,4 -methylenedioxybenzoyl)-3-carboxypyridine and 2-carboxy-3-(3,4-methylenedioxybenzoyl)pyridine;
2-(3-methoxybenzoyl)-3-carboxypyridine and 2-carboxy-3-(3-methoxybenzoyl)pyridine;
2-benzoyl-3-carboxypyridine and 2-carboxy-3-benzoylpyridine; and
2-benzoyl-3-carboxypyridine and 2-carboxy-3-benzoylpyridine.

Example 21

Preparation of 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine

21A. Formula II, Where X is carbon and Y is nitrogen, R^3 is chloro, and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 13A and substituting for 2-(3-chlorobenzoyl)-3-carboxypyridine with 2-carboxy-3-(3-chlorobenzoyl)pyridine there is obtained 2-methoxy-carbonyl-3-(3-chlorobenzoyl)pyridine.

21B. Formula II, Where X is carbon and Y is nitrogen.

By following the procedures of Example 21A and substituting for 2-carboxy-3-(3-chlorobenzoyl)pyridine with the following:

2-carboxy-3-(3-methoxybenzoyl)pyridine;
2-carboxy-3-(4-methoxybenzoyl)pyridine;
2-carboxy-3-(4-methylbenzoyl)pyridine;
2-carboxy-3-(3-bromobenzoyl)pyridine;
2-carboxy-3-(3-methoxycarbonylbenzoyl)pyridine;
2-carboxy-3-(4-methoxycarbonylbenzoyl)pyridine;
2-carboxy-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-carboxy-3-(4-carboxybenzoyl)pyridine;
2-carboxy-3-(4-carbamoylbenzoyl)pyridine;
2-carboxy-3o(4-N,N-dimethylcarbamoylbenzoyl)pyridine; and
2-carboxy-3-(3,4-methylenedioxybenzoyl)pyridine;

there are obtained the following compounds:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl)pyridine; and
2-methoxycarbonyl-3-(3,4-methylenedioxybenzoyl)pyridine.

Example 22

Preparation of 5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one

22A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^3 is chloro and R^1, R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 14A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine there is obtained 5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one.

22B. Preparation of Other Compounds of Formula II Where X is carbon, Y is nitrogen, Z is oxygen.

By following the procedures of EXample 22A and substituting for 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl)pyridine; and
2-methoxycarbonyl-3-(3,4-methylenedioxybenzoyl)pyridine;

there are obtained the following compounds:

5 -(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
5-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]-pyridazin-8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-8-one.

Example 23

Preparation of 5-(3-chlorophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one

23A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is phenyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 15A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine there is obtained 5-(3-chlorophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one.

23B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 23A and substituting for 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4- N,N-dimethylcarbamoylbenzoyl)pyridine; and
2-methoxycarbonyl -3-(3,4-methyl enedioxybenzoyl)pyridine;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-one;
5-(4-methoxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-one;
5-(4-methylphenyl)-7-phenylpyrido[2,3-d]pyridazin-one;
5-(3-bromophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-one;
5-(4-carbamoylphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-7-phenylpyrido-[2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one.

Example 24

Preparation of 5-(3-chlorophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one

24A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 16A and substituting for 2-(3-chlorobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine there is obtained 5-(3-chlorophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one.

24B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 24A and substituting for 2-methoxycarbonyl-3-(3-chlorobenzoyl)-pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl)pyridine; and
2-methoxycarbonyl-3-(3,4-methylenedioxybenzoyl)pyridine;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-one;
5-(4-methoxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-one;
5-(4-methylphenyl)-7-benzylpyrido[2,3-d]pyridazin-one;
5-(3-bromophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-7-benzyl-pyrido[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(4- N,N -dimethylcarbamoylphenyl)-7-benzylpyrido-[2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one.

Example 25

Preparation of 5-(3-chlorophenyl)-7 - ethylpyrido[2,3-d]pyridazin-8-one

25A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is ethyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 17A and substituting for 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one with 5-(3-chlorophenyl)pyrido[2,3-d]pyridazin-8-one there is obtained 5-(3-chlorophenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one.

25B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 25A and substituting for 5-(3 - chlorophenyl)-pyrido[2,3-d]pyridazin-8-one with the following:

5-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4- methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-one;
5-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d]-pyridazin -8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4- N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-8-one;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-7-ethylpyrido-[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-7-ethylpyrido-[2,3-d] pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one.

Example 26

Preparation of 5-(3 - chlorophenyl)-7-(2-propyl) pyrido[2,3-d]pyridazin-8-one

26A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 2-propyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 18A and substituting for 8-(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one with 5-(3- chlorophenyl) pyrido[2,3-d]pyridazin-8-one there is obtained 5-(3-chlorophenyl)-7-(2-propyl) pyrido[2,3-d]-pyridazin-8-one.

26B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 26A and substituting for 5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one with the following:

5-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)- pyrido[2,3-d]-pyridazin-8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4- N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin -8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin -8-one;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-(2-propyl)pyrido[2,3-d]-pyridazin -8-one;
5-(4-methoxyphenyl)-7-(2-propyl)pyrido[2,3-d]-pyridazin-8-one;
5-(4-methylphenyl)-7-(2-propyl)pyrido[2,3-d]-pyridazin-8-one;
5-(3-bromophenyl)-7-(2-propyl)pyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-(2-propyl)pyrido-[2,3-d] pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-(2-propyl) pyrido-[2,3-d] pyridazin-8-one;
5-(3 -methoxycarbonyl -4-methylphenyl)-7-(2-propyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-(2-propyl) pyrido[2,3-d]-pyridazin-8-one;
5-(4-carbamoylphenyl)-7-(2-propyl) pyrido[2,3-d]-pyridazin-8-one;
5-(4- N,N-dimethylcarbamoylphenyl)-7-(2-propyl)-pyrido [2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-(2-propyl)pyrido-[2,3-d] pyridazin-8-one.

Example 27

Preparation of 5-(3-chlorophenyl)-7-(4-pyridylmethyl)pyrido[2,3-d]pyridazin-8-one

27A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 4-pyridylmethyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 19A and substituting for 8-(3 -chlorophenyl)pyrido[2,3-d]pyridazin-5-one with 5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one there is obtained 5-(3- chlorophenyl)-7-(4-pyridomethyl)pyrido [2,3-d]-pyridazin-8-one.

27B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 27A and substituting for 5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-one with the following:

5-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d] pyridazin-8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-8-one;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-(4-pyridylmethyl)pyrido-[2,3-d] pyridazin-8-one;
5-(4-methoxyphenyl)-7-(4-pyridylmethyl)pyrido-[2,3-d] pyridazin-8-one;
5-(4-methylphenyl)-7-(4-pyridylmethyl)pyrido-[2,3-d] pyridazin-8-one;
5-(3-bromophenyl)-7-(4-pyridylmethyl)pyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-(4-pyridylmethyl)-pyrido [2,3-d]pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-(4-pyridylmethyl)-pyrido [2,3-d]pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-7-(4-pyridylmethyl)pyrido[2,3-d]pyridazin-8-one;

5-(4-carboxyphenyl)-7-(4-pyridylmethyl)pyrido -[2,3-d] pyridazin-8-one;
5-(4-carbamoylphenyl)-7-(4-pyridylmethyl)pyrido-[2,3-d] pyridazin-8-one;
5-(4- N,N-dimethylcarbamoylphenyl)-7-(4-pyridylmethyl) pyrido[2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-(4-pyridylmethyl)-pyrido [2,3-d]pyridazin-8-one.

EXAMPLE 28

Preparation of 2-Methoxycarbonyl-3-benzoylpyridine

28A. Formula II, Where X is carbon, Y is nitrogen and R^2, R^3, R^4, $R^5$and R^6 are hydrogen.

A solution of 2,3-pyridyldicarboxylic anhydride (10 g, 67.1 moles) in dry benzene (80 ml, 888 moles) was stirred at 50°–55° C. while aluminum chloride ($AlCl_3$, 20 g, 148 moles) was gradually added. The mixture was refluxed for 18 hours. The reaction mixture was cooled and poured into 250 ml of 5% aqueous HCl and extracted with ethyl acetate. The ethyl acetate was removed and the residue was dried under vacuum. The residue was dissolved in 500 ml methanol and 4 ml concentrated H_2SO_4 (67.1 moles) was added. The solution was refluxed at 80° C. for 24 hours. The mixture was cooled, neutralized with saturated sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate extract was washed with saturated brine and dried over sodium sulfate. The solution was filtered and the ethyl acetate removed under vacuum to yield 2-methoxycarbonyl-3-benzoylpyridine (7.92 g, 32.8 moles, 49% crude yield). The product was purified by column chromatography eluting with 5% ethyl acetate in methylene chloride to give after crystallization from ether the purified product (5.61 g, 23 moles, 35% yield), mp 85°–86° C.

Example 29

Preparation of 2-(3-nitrobenzoyl)-3 -methoxycarbonylpyridine

29A. Formula II, Where X is nitrogen, Y is carbon, R^3 is nitro, and R^2, R^4, R^5 and R^6 are hydrogen.

2-Benzoyl-3-methoxycarbonylpyridine (3.86 g, 16.0 moles) was dissolved in H_2SO_4 (10 ml) to which a mixture of HNO_3 (0.88 ml) and H_2SO_4 (2.0 ml) was added dropwise. The reaction mixture was stirred for 30 minutes and poured onto ice. The solution was stirred for 60 minutes, basified to pH 7.8 with saturated sodium bicarbonate and extracted with ethyl acetate. The organic ethyl acetate solution was washed with brine and dried over magnesium sulfate. The solution was filtered and the solvent removed yielding 4.49 g of the crude product, 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine.

29B. Formula II, Where X is carbon, Y is nitrogen, R^3 is nitro, and R^2, R^4, R^5 and R^6 are hydrogen.

By following the procedure of Example 29A and substituting 2-methoxycarbonyl-3-benzoylpyridine for 2-benzoyl-3-methoxycarbonylpyridine there is obtained 2-methoxycarbonyl-3-(3-nitrobenzoyl)pyridine.

Example 30

Preparation of 5-(3-nitrophenyl)-pyrido[2,3-d] pyridazin-8-one

30A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^3 is nitro and R^1, R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 5A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-nitrobenzoyl)pyridine, as prepared according to the procedure of Example 29B, there is obtained 5-(3-nitrophenyl)- pyrido[2,3-d]pyridazin-8-one.

30B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 30A and substituting for 2-methoxycarbonyl-3-(3-nitrobenzoyl)pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl) pyridine; and
2-methoxycarbonyl-3-(3,4-methylenedioxybenzoyl)-pyridine;

there are obtained the following compounds:

5-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)- pyrido[2,3-d]pyridazin-8-one;
5-(3- chlorophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d] pyridazin-8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-8-one.

Example 31

Preparation of 5-(3-nitrophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one

31A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is phenyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 6A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-nitrobenzoyl) pyridine there is obtained 5-(3- nitrophenyl)-7-phenylpyrido[2,3-d] pyridazin-8-one.

31B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 31A and substituting for 2-methoxycarbonyl-3-(3-nitrobenzoyl)pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;

2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl) pyridine; and
2- methoxycarbonyl-3-(3,4-methylenedioxybenzoyl) pyridine;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(3-chlorophenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-phenylpyrido[2,3-d]-pyridazin -8-one;
5-(4-methoxycarbonylphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonyl -4-methylphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-phenylpyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-7-phenylpyrido-[2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-phenylpyrido[2,3-d]-pyridazin-8-one.

Example 32

Preparation of 5-(3-nitrophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one

32A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is benzyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 7A and substituting for 2-(3-nitrobenzoyl)-3-methoxycarbonylpyridine with 2-methoxycarbonyl-3-(3-nitrobenzoyl) pyridine there is obtained 5-(3-nitrophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one.

32B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 32A and substituting for 2-methoxycarbonyl-3-(3-nitrobenzoyl)pyridine with the following:

2-methoxycarbonyl-3-(3-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methoxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(3-bromobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-chlorobenzoyl)pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(4-methoxycarbonylbenzoyl) pyridine;
2-methoxycarbonyl-3-(3-methoxycarbonyl-4-methylbenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carboxybenzoyl)pyridine;
2-methoxycarbonyl-3-(4-carbamoylbenzoyl) pyridine;
2-methoxycarbonyl-3-(4-N,N-dimethylcarbamoylbenzoyl) pyridine; and
2-methoxycarbonyl-3-(3,4-methylenedioxybenzoyl) pyridine;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(3-chlorophenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(3-methoxycarbonyl -4-methylphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(4-carboxyphenyl)-7-benzylpyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one;
5-(4-N,N-dimethylcarbamoylphenyl)-7-benzylpyrido-[2,3-d]pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-7-benzylpyrido[2,3-d]-pyridazin-8-one.

Example 33

Preparation of 5-(3-nitrophenyl)-7 -ethylpyrido[2,3-d]pyridazin-8-one

33A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is ethyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 8A and substituting for 8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one with 5-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-8-one there is obtained 5-(3-nitrophenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one.

33B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 33A and substituting for 5-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-8-one with the following:

5-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)- pyrido[2,3-d]pyridazin-8-one;
5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonyl-4-methylphenyl)-pyrido[2,3-d] pyridazin-8-one;
5-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-8-one;
5-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-8-one
5-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and
5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-8-one;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(4-methoxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(4-methylphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(3-bromophenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(3-chlorophenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;
5-(3-methoxycarbonylphenyl)-7-ethylpyrido[2,3-d] pyridazin-8-one;

5-(4-methoxycarbonylphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one;

5-(3-methoxycarbonyl-4-methylphenyl)-7-ethylpyrido-[2,3-d]pyridazin-8-one;

5-(4-carboxyphenyl)-7-ethylpyrido[2,3-d]pyridazin-8-one;

5-(4-carbamoylphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one;

5-(4-N,N-dimethylcarbamoylphenyl)-7-ethylpyrido-[2,3-d]pyridazin-8-one; and

5-(3,4-methylenedioxyphenyl)-7-ethylpyrido[2,3-d]-pyridazin-8-one.

Example 34

Preparation of 5-(3-nitrophenyl)-7(4-pyridylmethyl) pyrido[2,3-d]pyridazin-8-one

34A. Formula I, Where X is carbon, Y is nitrogen, Z is oxygen, R^1 is 4-pyridylmethyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen.

Following the procedures of Example 9A and substituting for 8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one with 5-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-8-one there is obtained 5-(3-nitrophenyl)-7-(4-pyridomethyl) pyrido[2,3-d]-pyridazin-8-one.

34B. Preparation of Other Compounds of Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

By following the procedures of Example 34A and substituting for 5-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-8-one with the following:

5-(3-methoxyphenyl)- pyrido[2,3-d]pyridazin-8-one;

5-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(3-bromophenyl)- pyrido[2,3-d]pyridazin-8-one;

5-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(3-methoxycarbonyl -4-methylphenyl)-pyrido[2,3-d]pyridazin-8-one;

5-(4-carboxyphenyl)- pyrido[2,3-d]pyridazin-8-one;

5-(4-carbamoylphenyl)- pyrido[2,3-d]pyridazin-8-one;

5-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]-pyridazin-8-one; and

5-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-8-one;

there are obtained the following compounds:

5-(3-methoxyphenyl)-7-(4-pyridomethyl)pyrido-[2,3-d]pyridazin-8-one;

5-(4-methoxyphenyl)-7-(4-pyridomethyl)pyrido-[2,3-d]pyridazin-8-one;

5-(4-methylphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]-pyridazin-8-one;

5-(3-bromophenyl)-7-(4-pyridomethyl)pyrido[2,3-d]-pyridazin-8-one;

5-(3-chlorophenyl)-7-(4-pyridomethyl)pyrido[2,3-d]-pyridazin-8-one;

5-(3-methoxycarbonylphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]pyridazin-8-one;

5-(4-methoxycarbonylphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]pyridazin-8-one;

5-(3-methoxycarbonyl -4-methylphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]pyridazin-8-one;

5-(4-carboxyphenyl)-7-(4-pyridomethyl)pyrido-[2,3-d]pyridazin-8-one;

5-(4-carbamoylphenyl)-7-(4-pyridomethyl)pyrido-[2,3-d]pyridazin-8-one;

5-(4-N,N-dimethyl carbamoylphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]pyridazin-8-one; and

5-(3,4-methylenedioxyphenyl)-7-(4-pyridomethyl)pyrido[2,3-d]pyridazin-8-one.

Example 35

Preparation of 6-(3-thienylmethyl)-8-(3-chlorophenyl) pyrido[2,3-d]pyridazin -5-one

35A. Formula I, Where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 3-thiophenemethyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

To a suspension of 8-(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one (0.21 g, 0.82 mmoles) in tetrahydrofuran (25 ml) was added 3-thiophenemethanol (0.085 ml, 0.9 moles) and triphenyl phosphine (0.32 g, 1.22 moles). To this mixture was added a solution of diisopropyl azodicarboxylate (0.24 ml, 1.22 moles) in tetrahydrofuran (5 ml) in a dropwise manner. The solution was stirred for 3 hours at room temperature. The solvent was removed and the residue chromatographed from hot methanol, yielding 0.15 g of 6-(3-thienylmethyl)-8-(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one, mp 165°–166° C.

35B. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen.

Following the procedures of Example 35A and substituting for 3-thiophenemethanol with the following:

4-(2-hydroxyethyl)morpholine;

3-pyridinepropanol;

2-(hydroxyethyl)pyridine;

1-(2-hydroxyethyl)pyrrolidine; and

1-(2-hydroxyethyl)-2-pyrrol idinone,

their were obtained the following compounds:

6-[4-(2-hydroxyethyl)morpholinyl]-8-(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one, mp 135° C.;

6-(3-pyridopropyl)-8-(3-chlorophenyl)pyrido[2,3-d]-pyridazin-5-one, mp 91° C.;

6-[2-(hydroxyethyl)pyridyl]-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one, mp 98° C.;

6-[1-(2-hydroxyethyl)pyrrolidyl]-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one, mp 149° C.; and

6-[1-(2-hydroxyethyl)-2-pyrrolidinonyl]-8(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one, mp 78° C.

35C. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, and Z is oxygen.

Following the procedures of Example 35A and substituting for 3-thiophenemethanol with the following:

furfuryl methanol;

4-(hydroxymethyl)imidazole;

2-thiophene methanol;

1-piperidine ethanol;

1-methyl-2-piperidine ethanol; and

1-methyl -3-piperidine ethanol,

there are obtained the following respective compounds:

6-(furfurylmethyl)-8-(3-chlorophenyl) pyrido[2,3-d]-pyridazin-5-one;

6-[4-(hydroxymethyl)imidazolyl]-8(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one;

6-(2-thienylmethyl)-8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin-5-one;

6-(1-piperidylethyl)-8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin -5-one;

6-(1-methyl-2-piperidylethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one; and

6-(1-methyl-3-piperidylethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

35D. Preparation of Other Compounds of Formula I Where X is nitrogen, Y is carbon, Z fs oxygen, and R^3 is nitro.

By following the procedures of Example 35A and substituting for 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one with 8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one there is obtained:

6-(3-thienylmethyl)-8-(3-nitrophenyl)pyrido[2,3-d]-pyridazin-5-one.

By following the procedures of Examples 35B and 35C and subtituting for 8-(3-chlorophenyl) pyrido[2,3-d] pyridazin-5-one with 8-(3-nitrophenyl) pyrido[2,3-d] pyridazin-5-one there are obtained the following compounds:

6-[4-(2-hydroxyethyl) morpholinyl]-8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one;

6-(3-pyridopropyl)-8-(3-nitrophenyl) pyrido[2,3-d]-pyridazin-5-one;

6-[2-(hydroxyethyl) pyridyl]-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;

6-[1-(2-hydroxyethyl) pyrrolidyl]-8-(3-nitrophenyl)-pyrido [2,3-d]pyridazin-5-one;

6-[1-(2-hydroxyethyl)-2-pyrrolidinonyl]-8(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one;

6-(furfurylmethyl)-8-(3-nitrophenyl) pyrido[2,3-d]-pyridazin-5-one;

6-[4-(hydroxymethyl)imidazolyl]-8-(3-nitrophenyl)pyrido [2,3-d]pyridazin-5-one;

6-(2-thienylmethyl)-8-(3-nitrophenyl)pyrido[2,3-d]-pyridazin-5-one;

6-(1-piperidylethyl)-8-(3-nitrophenyl)pyrido-[2,3-d] pyridazin-5-one;

6-(1-methyl-2-piperidylethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one; and

6-(1-methyl-3-piperidylethyl)-8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one.

Example 36

Preparation of 6-(4-pyridylmethyl)-8-(3-nitrophenyl)pyrido-[2,3-d]pyridazin-5-one hydrochloride

To a suspension of 6-(4-pyridylmethyl)-8-(3-nitrophenyl) pyrido[2,3-d]pyridazin-5-one in methanol is added 5% HCl/MeOH in a dropwise manner with stirring until a clear solution is obtained. The solvent is stripped off. The resulting solid is triturated with ethyl ether. The product is filtered off and dried under vacuum yielding 6-(4-pyridylmethyl)-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one hydrochloride.

In a similar manner, all compounds of Formula I may be converted to the acid addition salts by treatment with the appropriate acid, for example, hydrobromic acid, sulfuric acid (giving the sulfate and bisulfate salts), nitric acid, phosphoric acid and the like.

Example 37

Prepared Compounds of Formula I

By following the procedures in Examples 1–36 the following compounds were made:

Formula I, where X is nitrogen, Y is carbon, and Z is oxygen.

R^3 is chloro, R^1 is 2-propyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 80° C.;

R^3 is chloro, R^1 is 4-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 152° C.;

R^3 is chloro, R^1 is N-ethylmorpholinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 135 ° C.;

R^3 is chloro, R^1 is N-ethylpyrrolidonyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 78° C.;

R^3 is chloro, R^1 is 3-thienylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 165° C.;

R^3 is chloro, R^1 is 4-methoxy-3-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 145° C.;

R^3 is chloro, R^1 is 3-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 165° C.;

R^3 is chloro, R^1 is n-butyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 119° C.;

R^3 is chloro, R^1 is methyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 180° C.;

R^3 is chloro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 111°–112° C.;

R^3 is chloro, R^1 is ethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 117° C.;

R^3 is chloro, R^1, R^2, R^4, R^5 and R^6 are hydrogen, melting point 268°–269° C.;

R^3 is chloro, R^1 is phenyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 144°–145° C.;

R^3 is chloro, R^1 is N-methylpiperidinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 142° C.;

R^3 is chloro, R^1 is 2-pyridylethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 98° C.;

R^3 is chloro, R^1 is 3-pyridylpropyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 91° C.;

R^3 is chloro, R^1 is phenylethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 108° C.;

R^3 is chloro, R^1 is cyclopentyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 93° C.;

R^3 is chloro, R^1 is cyclohexyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 98° C.;

R^3 is chloro, R^1 is N-ethylpyrrolidinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 149° C.;

R^3 is chloro, R^1 is n-propyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 93° C.;

R^3 is chloro, R^1 is t-butyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 105° C.;

R^3 is chloro, R^1 is N-methylmorpholinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 161° C.;

R^3 is chloro, R^1 is 2-methylaminoethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 90° C.

R^3 is chloro, R^1 is cyclopentylmethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 125° C.

R^3 is chloro, R^1 is 2-thienylmethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 175° C.

R^3 is chloro, R^1 is furfuryl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 164° C.

R^3 is chloro, R^1 is 4-pyridyl-N-oxide-methyl and R^2, R^4, and R^6 are hydrogen, melting point 175°–176° C. (recryst. from MeOH).

R^3 is chloro, $R^1$is dimethylaminoethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 90° C.

R^3 is fluoro, R^1 is 4-pyridylmethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 158° C.

R^3 is fluoro, R^1 is 3-pyridinylmethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 151° C.

R^3 is fluoro, R^1 is ethyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 117°–118° C.

R^3 is fluoro, R^1 is benzyl and R^2, R^4, R^5 and R^6 are hydrogen, melting point 130°–131° C.

R^3 is nitro, R^1 is 4-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 172° C.;

R^3 is nitro, R^1 is 3-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 190° C.;

R^3 is nitro, R^1 is 3-thienylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 175° C.;

R^3 is nitro, R^1 is phenyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 167°–168° C.;

R^3 is nitro, R^1 is ethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 138°–139° C.;

R^3 is nitro, R^1, R^2, R^4, R^5 and R^6 are hydrogen, melting point 302°–304° C.;

R^3 is nitro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 141° C.;

R^3 is nitro, R^1 is cyclopentylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 88° C.;

R^3 is nitro, R^1 is dimethylaminoethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 108° C.;

R^3 is nitro, R^1 is 2-tetrahydrofuranylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 109° C.;

R^3 is nitro, R^1 is 3-tetrahydrofuranylmethyl, R^2, R^4, $R^5$and R^6 are hydrogen, melting point 76° C.;

R^3 is nitro, R^1 is cyclopropylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 101° C.;

R^3 is nitro, R^1 is 4-pyridyl-N-oxide-methyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 208° C.;

R^3 is nitro, R^1 is 2-propyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 126° C.;

R^3 is nitro, R^1 is 1,2-methylenedioxybenzene-5-methyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 199° C.;

R^3 is nitro, R^1 is 2-bromoethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 163° C.;

R^3 is nitro, R^1 is 2-fluoroethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 167° C.;

R^3 is nitro, R^1 is 2-chloroethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 166° C.;

R^3 is nitro, R^x is 3-methylcyclobutyl-3-methyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 159° C.;

R^3 is nitro, R^l is 2-cyanoethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 145° C.;

R^5 is nitro, R^1 is 3-methyl-3-butenyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 97° C.;

R^3 is nitro, R^1 is methylthioethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 96° C.;

R^3 is nitro, R^1 is 3-methyl-2-butenyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 119° C.;

R^3 is nitro, R^1 is methoxyethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 135° C.;

R^3 is nitro, R^1 is methylthiopropyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 71° C.;

R^3 is nitro, R^1 is 3,4-dichlorophenyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 168° C.;

R^3 is nitro, R^1 is 2-hydroxyethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 155° C.;

R^3 is nitro, R^1 is 2-thienylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 173° C.;

R^3 is nitro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 141° C.;

R^3 is nitro, R^1 is 1-pyrrolidinylethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 149° C.;

R^3 is nitro, R^1 is furfuryl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 159° C.;

R^3 is nitro, R^1 is 2-acetoxyethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 116° C.;

R^3 is trifluoromethyl, R^1 is cyanoethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 133° C.;

R^3 is trifluoromethyl, R^1 is ethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 116° C.;

R^3 is trifluoromethyl, R^1 is 3-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 142° C.;

R^3 is trifluoromethyl, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 137° C.;

R^3 is trifluoromethyl, R^1 is n-butyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 103° C.;

R^3 is cyano, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 158° C.;

R^3 is methyl, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 88° C.;

R^3 is methoxycarbonyl, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 128°–130° C.;

R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 169°–170° C.;

R^1 is 4-pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 175°–176° C.;

R^1, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 230°–231° C.;

R^1 is ethyl, R^3, R^2, R^4, R^5 and R^6 are hydrogen, melting point 114°–115° C.;

R^1 is methoxycarbonylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 97° C.;

R^1 is ethyl, R^4 is fluoro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 142° C.;

R^1 is α-methylbenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 137° C.;

R^1 is α-methylcyclopropylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 99° C.;

R^1 is 4-(3,5-dimethylisoxazolyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 177° C.;

R^1 is benzyl, R^4 is fluoro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 136° C.;

R^1 is 2-cyanoethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 141° C.;

R^1 is benzyl, R^3 and R^4 are chloro, R^2, R^5 and R^6 are hydrogen, melting point 145° C.;

R^1 is ethyl, R^3 and R^4 are chloro, R^2, R^5 and R^6 are hydrogen, melting point 114° C.;

R^1 is isopropyl, R^4 is fluoro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 155° C.;

R^1 is propanoyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 203° C.;

R^1 is hydrogen, R^3 and R^4 are chloro, R^2, R^5 and R^6 are hydrogen, melting point 272° C.;

R^1 is 4-pyridylmethyl, R^3 and R^4 are chloro, R^2, R^5 and R^6 are hydrogen, melting point 162° C.;

R^1 is 2-(1,3-dioxolanyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 129° C.;

R^1 is 4-pyridylmethyl, R^4 is fluoro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 144° C.;

R^1 is methoxycarbonylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 128° C.;

R^1 is α-methylcyclopropylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 76° C.;

R^1 is methoxycarbonylpropyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 95° C.;

R^1 is isopropyl, R^3 and R^4 are chloro, R^2, R^5 and R^6 are hydrogen, melting point 133° C.;

R^1 is 4-pyridylmethyl, R^4 is chloro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 162° C.;

R^1 is benzyl, R^4 is chloro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 133° C.;

R^1 is 3-(2-chloropyridyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 177° C.;

R^1 is 3-pyridonylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 258° C.;

R^1 is methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 188° C.;

R^1 is 2-chloro-6-methyl-4-pyridylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 157° C.;

R^1 is cyclopropylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 146° C.;

R^1 is 2-trifluoroethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 153° C.;

R^1 is allyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 99° C.;

R^1 is ethyl, R^4 is chloro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 120° C.;

R^1 is 1-methyl-3-pyridonylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 171° C.;

R^1 is 5-(4-methylthiazolyl)ethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 158° C.;

R^1 is α-methylbenzyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 99° C.;

R^1 is 2-chloro-6-methyl-4-pyridylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 192° C.;

R^1 is 3-methylpyridyl N-oxide, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 222° C.;

R^1 is 1-methyl-3-pyridonylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 214° C.;

R^1 is 3-pyridonylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 233° C.;

R^1 is (±)tetrahydro-3-furanmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 118° C.;

R^1 is 2-methoxy-5-pyridylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 145° C.;

R^1 is (±)tetrahydro-2-furanmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 113° C.;

R^1 is 5-(4-methylthiazolyl)ethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 141° C.;

R^1 is 2-chloro-4-pyridylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 163° C.;

R^1 is 2-methoxy-5-pyridylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 141° C.;

R^1 is isopropyl, R^4 is chloro, R^2, R^3, R^5 and R^6 are hydrogen, melting point 132° C.;

R^1 is propargyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 162° C.;

R^1 is carboxymethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 204° C.;

R^1 is 8-(6-fluorobenzo-1,3-dioxanyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 161° C.;

R^1 is 3-[5-(4-methoxyphenyl)-1,2,4-oxadiazolyl)methyl], R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 168° C.;

R^1 is carboxybutyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 152° C.;

R^1 is 1-(2-methyl-5 -nitroimidazolyl)ethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 184° C.;

R^1 is 4-[2-(4-chlorophenyl)thiazolyl]methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 206° C.;

R^1 is 4-(2-methylthiazolyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 170° C.;

R^1 is 4-nitrobenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 205° C.;

R^1 is 3-(5-phenyl-1, 2,4-oxadiazolyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 170° C.;

R^1 is 4-[2-(4-methoxybenzyl)thiazolyl]methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, 143° C.;

R^1 is 3-(1,2,4-oxadiazolyl)methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 116° C.;

R^1 is carboxypropyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 147° C.;

R^1 is 2-furanylmethyl, R^3 is methoxy, R^2, R^4, R^5 and R^6 are hydrogen, melting point 136° C.;

R^1 is methoxycarbonylbutyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 76° C.;

R^1 is 2-thienylmethyl, R^3 is methoxy, R^2, R^4, R^5 and R^6 are hydrogen, melting point 113° C.;

R^1 is 2-methyloxiranemethyl, R^3 is nitro, R^2, R^4, R^5 and are hydrogen, melting point 107° C.;

R^1 is 3-[5-(3,5-dimethylisoxazoyl-4-yl)-1,2,4,-oxadiazolyl]methyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 190° C.;

R^1 is 4-fluorobenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 155° C.;

R^1 is 4-methoxybenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 205° C.;

R^1 is 2-methoxybenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 189° C.;

R^1 is 3-methoxybenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 162° C.;

R^1 is 3-fluorobenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 139° C.;

R^1 is 4-methoxybenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 155° C.;

R^1 is 4-chlorobenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 177° C.;

R^1 is 3,5-dichlorobenzyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 189° C.; and

R^1 is 2-norboranylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, melting point 153° C.

Formula I, Where X is carbon, Y is nitrogen, and Z is oxygen.

R^1 is phenyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, having a melting point of 221°–222° C.;

R^1, R^2, R^3, R^4, R^5 and R^6 are hydrogen, having a melting point of 232°–233° C.;

R^1 is ethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, having a melting point of 164°–165° C.;

R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, having a melting point of 210°–211° C.; and

R^1 is 4-pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, having a melting point of 230°–231° C.

Formula I, Where X is carbon, Y is carbon, and Z is oxygen.

R^3 is chloro, R^1 is n-butyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 100° C.;

R^1 is n-butyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 105° C.;

R^1 is n-pentyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 93 ° C.;

R^3 is chloro, R^1 is 4-pyridylmethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 131° C.;

R^3 is chloro, R^1 is ethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 104° C.;

- R^1 is methyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 162° C.;
- R^3 is chloro, R^1 is cyclopentyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 139° C.;
- R^3 is chloro, R^1 is 2-propyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 85° C.;
- R^1 is n-propyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 104° C.;
- R^3 is trifluoromethyl, R^1 is methyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 136° C.;
- R^3 is nitro, R^1 is ethyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 161° C.;
- R^3 is nitro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 150° C.;
- R^3 is chloro, R^1 is hexyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 123° C.;
- R^3 is chloro, R^1 is N-methylmorpholinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 157° C.;
- R^3 is trifluoromethyl, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 54° C.;
- R^3 is chloro, R^1 is N-methylpiperidinyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 143° C.;
- R^1 is 2-propyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 128° C.;
- R^3 is chloro, R^1 is t-butyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 105° C.;
- R^3 is chloro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 137° C.;
- R^1, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 227°–228° C.;
- R^1 is benzyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 175°–176° C.;
- R^1 is ethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 120°–121° C.; and
- R^1 is 4-pyridylmethyl, R^2, R^3, R^4, R^5 and R^6 are hydrogen, melting point 201°–203° C.

Formula I, where X is nitrogen, Y is carbon, and Z is sulfur.

- R^3 is chloro, R^1 is benzyl, R^2, R^4, R^5 and R^6 are hydrogen, melting point 132° C.;

Example 38

Preparation of 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl) pyrido[2,3-d]pyridazin-5-one

38A. Formula I, where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is chloro and R^2, R^4, R^5 and R^6 are hydrogen.

To a suspension of 8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (0.50 g, 1.94 moles), 4-pyridylcarbinol N-oxide (0.27 g, 2.13 moles), triphenylphosphine (0.76 g, 2.9 moles) in tetrahydrofuran (50 ml) was added diisopropylazodicarboxylate (0.57 ml, 2.9 moles) in a dropwise fashion. The suspension was stirred under nitrogen for 18 hours at room temperature. The solvent was removed and the product purified by chromatography, eluted with 100% ethylacetate and 10% methanol/ethylacetate to yield 0.382 g of 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)pyrido[2,3-d]pyridazin-5-one (54%), mp 99° C.

38B. Preparation of Other Compounds of Formula I.

By following the procedures of Example 48A and substituting for 8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

- 8-(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-nitro-4-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-nitro -4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(3-methoxycarbonyl -4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
- 8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
- 8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]-pyridazin-5-one,

there are obtained the following compounds:

- 6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxyphenyl)-pyrido-[2 ,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-methoxyphenyl)-pyrido-[2 ,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-methylphenyl)-pyrido-[2 ,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-bromophenyl)-pyrido-[2 ,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitro-4-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitro-4-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxycarbonylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-methoxycarbonylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxycarbonyl-methylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-carboxyphenyl)-pyrido -[2,3-d]pyridazin -5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-carbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one;
- 6-(4-pyridyl-N-oxide-methyl)-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido-[2,3-d]pyridazin-5-one; and
- 6-(4-pyridyl-N-oxide-methyl)-8-(3,4-methylenedioxyphenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 39

Preparation of 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)pyrido[2,3-d]pyridazin-5-one

39A. Formula I, where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 4-pyridyl-N-oxide-methyl, R^3 is nitro and R^2, R^4, R^5 and R^6 are hydrogen.

To a solution of 8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one (0.40 g, 1.5 moles), 4-pyridylcarbinol N-oxide (0.205 g, 1.64 moles), triphenylphosphine (0.587 g, 2.24 moles) in tetrahydrofuran (50 ml) was added diisopropylazodicarboxylate (0.44 ml, 2.24 moles) in a dropwise fashion. The suspension was stirred under nitrogen for 18 hours at room temperature. The solvent was removed and the product purified by chromatography, i.e., eluted with 100% ethylacetate and 10% methanol/ethylacetate. The fractions were combined, the solvent removed yielding 0.390 g of product which was recrystallized from methanol to give 0.237 g of 6-(4-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one (42.1%), mp 208° C.

39B. Preparation of Other Compounds of Formula I

By following the procedures of Example 49A and substituting for 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

8(3-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-bromophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro -4-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-nitro-4-methylphenyl)-pyrido[2,3d]pyridazin-5-one;
8-(3-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-methoxycarbonylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(3-methoxycarbonyl -4-methylphenyl)-pyrido[2,3-d]-pyridazin-5-one;
8-(4-carboxyphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-carbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one;
8-(4-N,N-dimethylcarbamoylphenyl)- pyrido[2,3-d]-pyridazin-5-one; and
8-(3,4-methylenedioxyphenyl)- pyrido[2,3-d]-pyridazin-5-one,

there are obtained the following compounds:

6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxyphenyl)-5 pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-methoxyphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-methylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-bromophenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-nitro-4-chlorophenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-nitro-4-methylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxycarbonylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-methoxycarbonylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(3-methoxycarbonyl-4-methylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-carboxyphenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-carbamoylphenyl)-pyrido -[2,3-d]pyridazin-5-one;
6-(4-pyridyl-N-oxide-methyl)-8-(4-N,N-dimethylcarbamoylphenyl)-pyrido[2,3-d]pyridazin-5-one; and
6-(4-pyridyl-N-oxide-methyl)-8-(3,4-methylenedioxyphenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 40

Preparation of 6-(n-butyl)-pyrrolo[3,4-b]-pyridine -5,7-dione

Formula 11, where X is nitrogen, Y is carbon and R^7 is n-butyl

A suspension of 2,3-pyridinyldicarboxylic anhydride (90 g, 603 moles) was prepared with 400 mL of ethyl acetate. The mixture was cooled to 3° C. and n-butylamine (61 mL, 610 moles) was added at such a rate that the temperature remained below 25° C. Following the addition, the cooling bath was removed and thionyl chloride (51 mL, 700 moles) was added at such a rate that the temperature remained below 50° C. The mixture was stirred for 1 hour. The resulting suspension was then cooled to 5° C. and dilute sodium hydroxide (240 mL of a 6M solution, 1.4 moles) was added at such a rate that the temperature remained below 35° C. The resulting layers were separated. The bulk of the ethyl acetate was removed by atmospheric distillation. Methanol (500 mL) was then added and the distillation continued until 250 mL of solution remained. The solution was allowed to cool during which time crystallization occured. Water (250 mL) was then added and the mixture aged for 18 hours. The product was isolated by filtration and washed twice with 200 mL of a 1:1 (v:v) mixture of water and methanol. After drying 115 g of product 6-(n-butyl)-pyrrolo[3,4-b]pyridine-5,7-dione was obtained.

Example 41

Preparation of 7-Hydroxy-7-phenyl-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one

Preparation of Formula 13, Where X Is Nitrogen, Y Is Carbon, R^7 Is n-butyl, and R^2, R^3, R^4, R^5, R^6 Are Hydrogen.

6-(n-Butyl)-pyrrolo[3,4-b]pyridine-5,7-dione(40.4 g, 198 mmoles) was dissolved in toluene (1 L) and cooled to −65° C. Phenylmagnesium chloride (100 mL, 2M in THF, 200 moles) was added over 15 minutes. The cooling bath was then removed and the mixture allowed to warm to −10° C. The reaction was quenched by addition of 25 mL of saturated ammonium chloride solution. The room temperature solution was diluted with 500 mL of ethyl acetate and 500 mL of water. The layers were separated. Removal of most of the organic solvent was accomplished by atmospheric distillation. When the remaining volume reaches 150 mL the mixture was cooled to below 80 C and 1 L of hexanes was added. The resulting crystalline mass was filtered and dried to give 50.6 g of 7-hydroxy-7-phenyl-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one.

Example 42

Preparation of 7-hydroxy-7-(3-nitrophenyl)-7H -furo[3,4-b]pyridin -5-one

Preparation of Formula 15, Where X Is Nitrogen, R^3 Is Nitro, And R^2, R^4, R^5, R^6 Are Hydrogen

7-Hydroxy-7-phenyl-6-(n-butyl)-6,7-dihydropyrrolo-[3,4-b]pyridin-5-one (47.15 g, 167 moles) was mixed with 250 mL of concentrated sulfuric acid (96%). The resulting mixture was placed in a cooling bath. Nitric acid (70%, 12 mL, 189 moles) was added at such a rate that the temperature remained below 45° C. The mixture was stirred for 30 minutes poured onto ice (500 g) and diluted with 1 L of water. The mixture was extracted twice with 700 mL of ethyl acetate. The organic solvent was removed yielding 7-hydroxy-7-(3-nitrophenyl)-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]-pyridin-5-one, which was used without further purification.

The 7-hydroxy-7-(3-nitrophenyl)-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one was mixed with 500 mL of water and 250 mL of concentrated hydrochloric acid was added. The mixture was heated under reflux for 20 hours. After cooling the mixture was diluted with 500 mL of water and the solid was removed by filtration. After washing the solid with water (500 mL) and methanol (200 mL) the material was dried to give 39.85 of 7-hydroxy-7-(3-nitrophenyl)-7H-furo[3,4-b]pyridin-5-one.

Example 43

Preparation of 8-(3-nitrophenyl)-pyrido-[2,3-d] pyridazin-5-one

Preparation of Formula I, Where X Is Nitrogen, Y Is Carbon, R^3 Is Nitro, And R^2, R^4, R^5, R^6 Are Hydrogen

7-Hydroxy-7-(3-nitrophenyl)-7H-furo[3,4-b]pyridin-5-one (38.65 g, 142 mmoles) was mixed with 800 mL of methanol. To this mixture was added 35 mL of anhydrous hydrazine. The resulting solution was then heated under reflux for 5 hours. After 17 hours of aging at room temperature the product was isolated by filtration. The crystalline mass was washed twice with methanol (250 mL) and dried, yielding 25.6 g of 8-(3-nitrophenyl)- pyrido[2,3-d] pyridazin-5-one.

Example 44

Preparation of 6-(4-Pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one

44A. Preparation Of Formula I, Where X Is Nitrogen, R^1 Is 4-Pyridylmethyl, R^3 Is Nitro, And R^2, R^4, R^5, And R^6 Are Hydrogen.

8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one (5.12 g, 19.1 moles), sodium hydride (50% in oil, 1 g, 20.1 moles) were mixed in 100 mL of 1-methyl-2-pyrrolidone and the mixture heated to 65° C. In a separatory funnel, 4-picolyl chloride hydrochloride (4.5 g, 27.4 mmoles), 20 mL of saturated potassium carbonate and 75 mL of toluene are mixed. After separation, the toluene solution was added to the reaction mixture dropwise over the period of 1 hour. The reaction mixture was diluted with water (250 mL) and extracted twice with ethyl acetate (200 mL). After concentrating the organic fractions, methanol and hexanes (75 mL each) were added and the mixture allowed to stir at room temperature for 17 hours. The material was filtered and dried to yield 5.2 g of 6-(4-pyridylmethyl)-8-(3-nitro-phenyl)-pyrido[2,3-d]pyridazin-5-one.

44B. Formula I Varying R^1

By following the procedures of Example 44A and substituting for 4-picolyl chloride hydrochloride with the following:

3-picolyl chloride hydrochloride;
2-picolyl chloride hydrochloride;
2-imidazoylmethyl chloride hydrochloride; or
2-imidazoylethyl chloride hydrochloride;

there are obtained the following compounds:

6-(3-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-imidazoylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one; or
6-(2-imidazoylethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one.

Example 45

Preparation of 6-(4-Pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one

45A. Preparation of Formula I, Where X Is Nitrogen, Y Is Carbon, R^1 Is 4-Pyridylmethyl, R^3 Is Nitro, And R^2, R^4, R^5, R^6 Are Hydrogen

6-(4-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one (4.2 g, 11.5 moles) was dissolved in 100 mL of methylene chloride. To this solution was added m-chloroperoxybenzoic acid (85%, 4.2 g, 20.7 moles). The solution was stirred for 17 hours. After partitioning between methylene chloride and potassium carbonate the organic layer was directly chromatographed using silica gel. The product was eluted with 20% methanol in ethyl acetate. After solvent removal, and filtering, recrystallization from methanol (200 mL) gave 3.2 g of 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

45B. Formula I Varying R^1

By following the procedures of Example 45A and substituting for 6-(4-pyridylmethyl)-8-(3-nitrophenyl)-pyrido [2,3-d]pyridazin-5-one with the following:

6-(3-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-imidazoylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one; or
6-(2-imidazoylethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]-pyridazin-5-one;

there are obtained the following compounds:

6-(3-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-pyridyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-imidazoyl-N-oxide-methyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5 -one; or
6-(2-imidazoyl-N-oxide-ethyl)-8-(3-nitrophenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 46

Preparation of 7-hydroxy-7-(3-chlorophenyl)-7H-furo[3,4-b]pyridin-5-one

Preparation Of Formula 19, Where X Is Nitrogen, Y Is Carbon, R^7 Is n-Butyl, R^3 Is Chloro And R^2, R^4, R^5, R^6 Are Hydrogen.

6-(n-Butyl)-pyrrolo[3,4-b]pyridine-5,7-dione (20 g, 98 mmole) was dissolved in toluene (500 mL) and cooled to −65° C. To this mixture was added 3-chlorophenylmagnesium bromide (prepared by heating under reflux 2.5 g magnesium metal, 20 g of 3-chlorobromobenzene and 100 mL of tetrahydrofuran for 1 hour) at such a rate that the temperature of the reaction remain below −40° C. After the addition was complete, the cooling bath was removed and the mixture was allowed to warm to 0° C. The reaction mixture was quenched with aqueous ammonium chloride (25 mL) diluted with ethyl acetate (250 mL) and water (250 mL). After separating the resulting 2 layers, the organic fraction was dried with sodium sulfate (50 g). Filtration, followed by solvent removal gave 7-hydroxy-7-(3-chlorophenyl)-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one as an oil, which was used in the next reaction without further purification.

To the 7-hydroxy-7-(3-chlorophenyl)-6-(n-butyl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one was added 500 mL of water, 250 mL of concentrated hydrochloric acid and the mixture was heated under reflux for 24 hours. The solution was then filtered, cooled and made basic with sodium hydroxide. An ethyl acetate extraction removed unhydrolyzed starting material and the pH of the resulting aqueous fraction was adjusted to pH 2 with hydrochloric acid. The resulting aqueous fraction was extracted twice with ethyl acetate (250 mL). The solvent was removed yielding 16 g of 7-hydroxy-7-(3-chlorophenyl)-7H-furo[3,4-b]pyridin-5-one, which was used without further purification.

Example 47

Preparation of 8-(3-chlorophenyl)-pyrido-[2,3-d] pyridazin-5-one

Preparation of Formula I, where X is nitrogen, R^1 is n-butyl, R^3 is chlorine, and R^2, R^4, R^5, R^6 are hydrogen

7-Hydroxy-7-(3-chlorophenyl)-7H-furo[3,4-b]pyridin-5-one was mixed with 100 mL of methanol. To this mixture was added 20 mL of anhydrous hydrazine. The mixture was heated under reflux for 2 hours at which time an additional 100 mL of methanol and 200 mL of xylenes were added. The mixture was heated under reflux for an additional 17 hours. Additional xylenes (200 mL) were added and 200 mL of solvent was distilled. Isolation of the product was accomplished by cooling the reaction mixture and filtration. The resulting crystalline mass was washed with toluene (200 mL) and dried to yield 14.2g of 8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 48

Preparation of 6-(4-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one

48A. Preparation of Formula I, Where X Is Nitrogen, Y Is Carbon, R^1 Is 4-Pyridylmethyl, R^3 Is Chloro, And R^2, R^4, R^5, R^6 Are Hydrogen.

8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one (14.2 g, 55 mole) and sodium hydride (50% in oil, 2.7 g, 55 mole) were mixed in 150 mL of 1-methyl-2-pyrrolidone and the mixture was heated to 55° C. In a separatory funnel, 4-picolyl chloride hydrochloride (11 g, 67 mmoles), 50 mL of saturated potassium carbonate and 150 mL of toluene were mixed. After separation, the toluene solution was added to the reaction mixture dropwise over the period of 30 minutes. The mixture was heated to 60° C. for an additional 30 minutes, cooled to room temperature and poured into 300 mL of water. The mixture was extracted twice with ethyl acetate (500 mL). After drying the organic fraction with sodium sulfate (50 g), filtering and removal of the solvent the crude product was crystallized from isopropyl acetate (300 mL). The material was filtered and dried to yield 10.5 grams of 6-(2-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one. An additional 4.6 grams of the product was obtained as a second crop by concentration of the filtrates and filtering the resulting crystalline mass.

48B. Formula I Varying R^1

By following the procedures of Example 48A and substituting for 4-picolyl chloride hydrochloride with the following:

3-picolyl chloride hydrochloride;
2-picolyl chloride hydrochloride;
2-imidazoylmethyl chloride hydrochloride; or
2-imidazoylethyl chloride hydrochloride;

there are obtained the following compounds:

6-(3-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one;
6-(2-imidazoylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one; or
6-(2-imidazoylethyl)-8-(3-chlorophenyl)- pyrido[2,3-d]-pyridazin-5-one.

Example 49

Preparation of 6-(4-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)- pyrido[2,3-d]pyridazin-5-one

49A. Preparation of Formula I, where X is nitrogen, Y Is Carbon, R^1 Is 4-Pyridylmethyl, R^3 Is Chloro, And R^2, R^4, R^5, R^6 Are Hydrogen.

6-(2-Pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one (6 g, 17.2 mole) was mixed with m-chloroperoxybenzoic acid (85%, 4.5 g, 22.1 moles) in methylene chloride (50 mL). The mixture was stirred for 2 hours at room temperature. Saturated sodium carbonate (25 mL) was added the resulting layers separated and the methylene chloride fraction dried with sodium sulfate (25 g). After filtration, the solvent was removed and the resulting solid crystallized from methanol (20 mL). Filtration and drying yielded 3.8 g of 6-(2-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

49B. Formula I Varying R^1

By following the procedures of Example 15A and substituting for 6-(2-pyridyl -N-oxide-methyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one with the following:

6-(3-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d] pyridazin-5-one;
6-(2-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d] pyridazin-5-one;
6-(2-imidazoylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one; or
6-(2-imidazoylethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]-pyridazin-5-one

there are obtained the following compounds:

6-(3-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-pyridyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one;
6-(2-imidazoyl-N-oxide-methyl)-8-(3-chlorophenyl)-pyrido [2,3-d]pyridazin-5-one; or
6-(2-imidazoyl-N-oxide-ethyl)-8-(3-chlorophenyl)-pyrido-[2,3-d]pyridazin-5-one.

Example 50

Preparation of 6-benzyl-8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin-5-thione

Formula I Where X Is Nitrogen, Y Is Carbon, Z Is Sulfur, R^1 Is Benzyl, R^3 Is Chloro and R^2, R^4, R^5 and R^6 Are Hydrogen

To a solution of 6-benzyl-8-(3-chlorophenyl)pyrido-[2,3-d]pyridazin-5-one (0.96 g, 2.76 mmoles) in toluene (100 mL) at room temperature under a nitrogen atmosphere was added solid 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's Reagent) (0.67 g, 1.66 mmoles). The reaction mixture was heated under reflux for 18 hours, and then cooled and concentrated. Chromatography on silica gel using ethyl acetate/hexane (1:4) gave 0.233 g of 6-benzyl-8-(3-chlorophenyl)pyrido[2,3-d] pyridazin-5-thione (23.2%), mp 132° C.

Example 51

Preparation of 8-(3-chlorophenyl)-6-(6-methoxy-3-pyridylmethyl)pyrido[2,3-d]pyridazin-5-one

Formula I, Where X Is Nitrogen, Y Is Carbon, Z Is Oxygen, R^1 is 6-Methoxy-3-pyridylmethyl, R^3 Is Chloro, And R^2, R^4, R^5, R^6 Are Hydrogen

To a suspension of 8-(3-chlorophenyl)pyrido-[2,3-d] pyridazin-5-one (0.515 g, 2.0 moles), 6-methoxy-3-hydroxymethyl pyridine (0.306g, 2.2 moles) and triphenylphosphine (0.792 g, 3.0 moles) in THF (50 mL) was added diisopropylazodicarboxylate (0.6mL, 3.0 moles). The reaction mixture was stirred for 18 hours at room temperature. The solvent was removed and the product was purified by chromatography (ethyl acetate/hexane 2:3) and crystallized from methanol to yield 0.588g of 8-(3-chlorophenyl)

-6-(6-methoxy-3-pyridylmethyl)pyrido[2,3-d]-pyridazin-5-one, mp 145° C.

Example 52

Preparation of 8-(3-chlorophenyl)-6-(3-pyridon-6-yl methyl)pyrido-[2,3-d]pyridazin-5-one

Formula I, where X is nitrogen, Y is carbon, Z is oxygen, R^1 is 3-pyridon-6-ylmethyl, R^3 is chloro, and R^2, R^4, R^5, R^6 are hydrogen.

To a solution of 8-(3-chlorophenyl)-6-(6-methoxy-3-pyridylmethyl)pyrido[2,3-d]pyridazin-5-one (0.566 g, 1.5 moles) in acetonitrile was added solid sodium iodide (0.224 g). The suspension was stirred for 30 min at room temperature, and then trimethylsilyl chloride (0.19 mL, 1.5 moles) was added. The reaction mixture was heated under reflux for 18 hours, then allowed to cool. Water (50 mL) was added. The solution was extracted with ethyl acetate, washed with 10% sodium thiosulfate and brine, dried over magnesium sulfate, filtered and concentrated. Chromatography with 10% methanol/ethyl acetate yielded 0.317 g of 8-(3-chlorophenyl)-6-(3-pyridon-6-ylmethyl)pyrido-[2,3-d]pyridazin-5-one, mp 233° C.

Example 53

Preparation of 8-(3-chlorophenyl)-6-(1-methyl-3-pyridon-6-ylmethyl)pyrido[2,3-d]pyridazin-5-one

Formula I, Where X Is Nitrogen, Y Is Carbon, Z Is Oxygen, R^1 Is 1-Methyl-3-pyridon-6-ylmethyl, R^3 Is Chloro, And R^2, R^4, R^5, R^6 Are Hydrogen

To a suspension of 8-(3-chlorophenyl)-6-(3-pyridon-6-ylmethyl)pyrido-[2,3-d]pyridazin-5-one (0.35 g, 0.96 moles) in DMF (15 mL) was added potassium carbonate (0.16 g, 1.15 mmoles). The suspension was stirred at room temperature for 1 hour under nitrogen, then methyl iodide (0.12 mL) was slowly added via syringe. The reaction mixture was heated under reflux for 18 hours, then allowed to cool. 1N HCl (35 mL) was added, the solid material collected, dried and chromatographed with ethyl acetate and then 5% methanol/ethyl acetate to give 0.055g of 8-(3-chloro-phenyl)-6-(1-methyl-3-pyridon-6-ylmethyl)pyrido[2,3-d]pyridazin-5-one, mp 171° C.

Example 54

Determination of Potency and Selectivity of Inhibitors for PDE IV

Preparation of Human Platelet Phosphodiesterase (PDE III)

Platelet high-affinity cAMP PDE (PDE III) was obtained from human blood in accordance with previously described procedures described in *Mol. Pharmacol.* 20:302–309, Alvarez, R., Taylor, A., Fazarri, J. J., and Jacobs, J. R. (1981).

Blood was collected into evacuated tubes containing EDTA (7.7 mM, final concentration). PRP was obtained by centrifuging the blood in polycarbonate tubes at 200×g for 15 min at 4° C. A platelet pellet was resuspended in a volume of buffer A (0.137 M NaCl, 12.3 mMTris-HCl buffer, pH 7.7, containing 1 mM $MgCl_2$. The hypotonically-lysed platelet suspension was centrifuged at 48,000×g for 15 min and the supernatant was saved. The pellets were frozen on dry ice and briefly thawed at 22° C. The supernatant was combined with the pellet fraction and the resulting suspension was centrifuged at 48,000×g for 30 min. The supernatant fraction was stored in 0.5 ml aliquots at −20° C. and used as the soluble PDE. Enzyme activity was adjusted to 10–20% hydrolysis after 10 minutes of incubation by dilution with 10mM cold Tris-HCl buffer, pH 7.7.

Preparation of Human Lymphocyte Phosphodiesterase (PDE IV)

Human B cell line (43D) was cultured at 37° C. in 7% CO_2 in RPMI 1640 with L-glutamine and 10% Nu-Serum. Prior to the assay ~1.5×10^8 cells were centrifuged at 1000 rpm for 10 minutes in a table top clinical centrifuge. The pellet was resuspended in 2–3 ml of 45 mMTris-HCl buffer, pH 7.4. The suspension was homogenized and centrifuged at 12,000×g 4° C. for 10 minutes. The supernatant was diluted to 28 ml with Tris-HCl buffer and used directly in the assay or stored at −20° C. The final concentration of DMSO in the PDE incubation medium was 1%. Nitraquazone was included in each assay (10 and 100 μM) as a reference standard.

Human Platelet cAMP Phosphodiesterase Assay

The phosphodiesterase incubation medium contained 10 mM Tris-HCl buffer, pH 7.7, 10 mM $MgSO_4$, 0.1–1 μM [^{3}H]-AMP (0.2 μCi) in a total volume of 1.0 ml. Following addition of the enzyme, the contents were mixed and incubated for 10 min at 30° C. The reaction was terminated by immersing the tubes in a boiling-water bath for 90 sec. After the tubes were cooled in an ice-water bath, 0.1 ml (100μg) of 5'-nucleotidase from snake venom (Crotalus atrox, Sigma V-7000) was added to each tube. The contents were mixed and incubated for 30 min at 30° C. The nucleotidase reaction was terminated by immersing the tubes in a boiling water bath for 60 sec. Labeled adenosine was isolated from alumina coles according to the method described in *Anal. Biochem.*, 52:505–516 (1973), Filburn, C. R., and Karn, J. Assays were performed in triplicate. Hydrolysis of cAMP ranged from 10–20%. Test compounds were dissolved in DMSO. The final concentration of DMSO in the phosphodiesterase assay was 1% when tested with compounds up to 0.1 mM. When tested at 1 mM the DMSO concentration was 10% and this activity was compared to control PDE activity in the presence of 10% DMSO.

Human Lymphocyte cAMP Phosphodiesterase Assay

The phosphodiesterase incubation medium contained 40 mM Tris-HCl buffer, pH 7.7, 0.1 mM $MgSO_4$, 3.75 mM mercaptoethanol, and 0.1–1.0 μM [^{3}H] cAMP (0.2 μCi) in a total volume of 1.0 ml. The reaction was performed and processed according to the procedure used (above) for human platelet PDE. The final concentration of DMSO was 1%.

The compounds of the present invention exhibit potency and selectivity as inhibitors of PDE IV when tested by this method.

Inhibition of Human Lymphocyte PDE IV

Compound I	IC_{50}:	0.00023 μM
Compound II	IC_{50}:	0.001 μM

Compound I is the compound of Formula I where X is nitrogen and Y is carbon, R^1 is 4-pyridylmethyl, R^3 is nitro, R^2, R^4, R^5 and R^6 are hydrogen, namely

6-(4-pyridylmethyl)-8-(3-nitrophenyl)-pyrido[2,3-d]pyridazin-5-one.

Compound II is the compound of Formula I where X is nitrogen and Y is carbon, R^1 is 4-pyridylmethyl, R^3 is chloro, R^2, R^4, R^5 and R^6 are hydrogen, namely

6-(4-pyridylmethyl)-8-(3-chlorophenyl)-pyrido[2,3-d]pyridazin-5-one.

Example 55

Determination of Immunosuppressive Activity Utilizing The Hemolytic Plaque Forming Cell Assay

This procedure is a modification of "The agar plaque technique for recognizing antibody producing cells," a procedure initially described by Jerne, et al. [*Cell-bound Antibodies*, Amos and Kaprowski editors (Wistar Institute Press, Philadelphia, 1963) p. 109].

Groups of 5–6 adult C3H female mice were sensitized with 1.25×10^8 sheep red blood cells (SRBC) and simultaneously treated with an oral dosage form of the test material in an aqueous vehicle. Animals in a control group receive the same volume of vehicle. Four days after SRBC inoculation, spleens are dispersed in glass homogenizers. The number of nucleated cells (WBC) is determined and the spleen cell suspension is mixed with SRBC, guinea pig complement and agar solution at 0.5% concentration. Aliquots of the above mixture (0.1 ml) are dropped on four separate quadrants of a Petri dish and are covered with cover slips. After two hours incubation at 37° C., areas of hemolysis around plaque-forming cells (PFC) are counted with a dissecting microscope. Total WBC/spleen, PFC/spleen and PFC/10^6WBC (PPM) are calculated for each mouse spleen. Geometric means of each treatment group are then compared with the vehicle-treated control group.

The compounds of the present invention show immunosuppressive activity when tested by this method.

Immunosuppressive Activity of Compounds I and II

	Immune PFC
	ED_{50}
Compound I	1 mg/kg
Compound II	3 mg/kg

Example 56

Determination of Immunosuppressive Activity Utilizing Responses of Human Peripheral Blood Lymphocytes to Mitogen

This procedure is a modification of a procedure initially described by Greaves, et al. ["Activation of human T and B lymphocytes by polyclonal mitogens," Nature, 248, 698–701 (1974)].

Human mononuclear cells (PBL) are separated from heparinized whole blood by density gradient centrifugation in Ficoll-Paque (Pharmacia). After washing, 5×10^4 cells/well are cultured in microtiter plates with minimal essential media supplemented with 1% human serum, gentamicin, sodium bicarbonate, 2-mercaptoethanol, glutamine, non-essential amino acids, and sodium pyruvate. The mitogen concanavalin A (Sigma) is used at a concentration of 2 μg/ml. Test materials are tested at concentrations between 10^{-4} and 10^{-10}M, by addition to the culture at time 0. Cultures are set up in quadruplicate and incubated at 37° C. in a humidified atmosphere with 5% CO_2 for 48 hours. A pulse of 1.0 μCi/well of ^{3}H-thymidine is added for the last 4 hours. Cells are collected on glass fiber filters with an automatic harvester and radioactivity is measured by standard scintillation procedures. The 50% inhibitory concentration ("IC_{50}") for mitogenic stimulation is determined graphically. The compounds of the present invention show immunosuppressive activity when testedby this method.

EFFECT OF COMPOUNDS ON MITOGENIC RESPONSE OF HUMAN PBL TO CONCANAVALIN IN A STIMULATION

	% Suppression	
Concentration	Compound I	Compound II
100 μM	100	100
10 μM	85	87
1 μM	80	83
0.1 μM	83	74

The % suppression represents test well counts minus background (wells with no concanavalin A) divided by controll well counts (wells with no compound) minus background times 100.

Example 57

Determination of Anti-Inflammatory Activity Utilizing Arachidonic Acid-Induced Ear Edema in the Mouse

This procedure is a modification of a procedure described by Young et al., *J. Invest. Derm.*, 82:367–371 (1984).

Female Charles River ICR mice 23–27 grams are administered 0.2 ml of test material. The mice are later challenged with 20 μl of arachidonic acid applied topically to the ear. One hour after challenge the weight of an 8 mm disc is determined. The mean increase in ear plug weight is calculated. Materials with anti-inflammatory activity inhibit the increase in ear plug weight.

Antiinflammatory Activity of Compounds I and II

	AAEE ED_{50}
Compound I	0.003 mg/kg
Compound II	0.009 mg/kg

The compounds of the present invention exhibit anti-inflammatory activity when tested by this method.

Example 58

Capsule Formulation

This example illustrates the preparation of a representative pharmaceutical formulation for oral administration containing an active compound of Formula I.

Ingredients	Quantity (mg/capsule)
Active compound	200
lactose, spray-dried	148
magnesium stearate	2

The above ingredients are mixed and introduced into a hard-shell gelatin capsule.

Example 59

Oral Formulation

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula I.

An suspension for oral administration is prepared having the following composition:

Ingredients	Quantity
Active compound	1.0 g
fumaric acid	0.5 g
sodium chloride	2.0 g
methyl paraben	0.1 g
granulated sugar	25.5 g
sorbitol (70% solution)	12.85 g
Veegum K (Vanderbilt Co.)	1.0 g
flavoring	0.035 mL
colorings	0.5 mg
distilled water	q.s. to 100 mL

Other compounds of Formula I, such as those prepared in accordance with Examples 1–45 can be used as the active compound in the preparation of the orally administrable formulations of this example.

Example 60

Tablet Formulation

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula I.

A tablet for oral administration is prepared having the following composition:

Ingredients	Quantity (mg/tablet)
Active compound	400
corn starch	50
lactose	145
magnesium stearate	5

The above ingredients are mixed intimately and pressed into single scored tablets.

Other compounds of Formula I, such as those prepared in accordance with Examples 1–45 can be used as the active compound in the preparation of the tablet formulations of this example.

Example 61

Injectable Formulation

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula I.

An injectable preparation is prepared having the following composition:

Ingredients	Quantity
Active compound	0.2 g
water (distilled, sterile)	q.s. to 20.0 mL

Other compounds of Formula I, such as those prepared in accordance with Examples 1–45 can be used as the active compound in the preparation of the injection administrable formulations of this example.

Example 62

Suppository Formulation

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula I.

A suppository totalling 2.5 grams is prepared having the following composition:

Ingredients	Quantity
Active compound	500 mg
Witepsol H-15*	q.s. to 2.5 g

*(triglycerides of saturated vegatable fatty acid; a product of Riches-Nelson, Inc., New York, N.Y.).

Other compounds of Formula I, such as those prepared in accordance with Examples 1–45 can be used as the active compound in the preparation of the suppository formulations of this example.

Other compounds of Formula I, such as those prepared in accordance with Examples 1–45 can be used as the active compound in the preparation of the injection administrable formulations of this example.

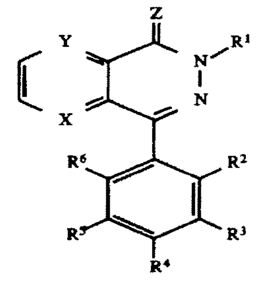
TOXICOLOGY	
SPECIES:	mice
COMPOUND:	compounds I, II
DOSAGE:	3 to 25 mg/kg/day
DURATION OF DOSING:	2 weeks
CONCLUSION:	no mortality occurred at any of the dose levels tested.

The Ames test (in vitro Salmonella mutagenicity assay) was negative.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. A compound of the formula:



wherein:

X is nitrogen and Y is carbon, or X is carbon and Y is nitrogen;

Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo lower alkyl (where the heterocyclo is selected from pyrrolidinyl, piperidinyl, N-methylpiperidinyl, or morpholinyl, or heteroaryl lower alkyl (where the heteroaryl is selected from 2-pyridyl, 3-pyridyl, 4-pyridyl, the N-oxides thereof, 2-thienyl, or 3-thienyl, each optionally substituted with halo or lower alkyl);

R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein X is nitrogen and Y is carbon.

3. The compound of claim 2 wherein R^1 is hydrogen.

4. The compound of claim 2 wherein R^1 is aralkyl.

5. The compound of claim 2 wherein R^1 is heterocyclo lower alkyl or heteroaryl lower alkyl.

6. The compound of claim 2 wherein R^1 is lower alkyl or cycloalkyl lower alkyl.

7. The compound of claim 2 wherein R^2, R^4, R^5 and R^6 are hydrogen and R^3 is chloro.

8. The compound of claim 7 wherein R^1 is hydrogen.

9. The compound of claim 7 wherein R^1 is aralkyl.

10. The compound of claim 7 wherein R^1 is benzyl.

11. The compound of claim 7 wherein R^1 is heterocyclo lower alkyl or heteroaryl lower alkyl.

12. The compound of claim 11 wherein R^1 is 4-pyridylmethyl.

13. The compound of claim 11 wherein R^1 is 3-pyridylmethyl.

14. The compound of claim 11 wherein R^1 is 3-thienylmethyl.

15. The compound of claim 7 wherein R^1 is lower alkyl or cycloalkyl lower alkyl.

16. The compound of claim 15 wherein R^1 is cyclopentylmethyl.

17. The compound of claim 15 wherein R^1 is 2-propyl.

18. The compound of claim 2 wherein R^2, R^4, R^5 and R^6 are hydrogen and R^3 is nitro.

19. The compound of claim 18 wherein R^1 is hydrogen.

20. The compound of claim 18 wherein R^1 is cyclopropylmethyl.

21. The compound of claim 18 wherein R^1 is aralkyl.

22. The compound of claim 21 wherein R^1 is benzyl.

23. The compound of claim 18 wherein R^1 is heterocyclo lower alkyl or heteroaryl lower alkyl.

24. The compound of claim 23 wherein R^1 is 4-pyridylmethyl.

25. The compound of claim 23 wherein R^1 is 3-pyridylmethyl.

26. The compound of claim 23 wherein R^1 is 3-thienylmethyl.

27. The compound of claim 18 wherein R^1 is lower alkyl or cycloalkyl lower alkyl.

28. The compound of claim 27 wherein R^1 is ethyl.

29. The compound of claim 2 wherein R^2, R^3, R^4, R^5 and R^6 are hydrogen.

30. The compound of claim 29 wherein R^1 is hydrogen.

31. The compound of claim 29 wherein R^1 is aralkyl.

32. The compound of claim 29 wherein R^1 is heterocyclo lower alkyl or heteroaryl lower alkyl.

33. The compound of claim 29 wherein R^1 is lower alkyl or cycloalkyl lower alkyl.

34. The compound of claim 1 wherein X is carbon and Y is nitrogen.

35. The compound of claim 34 wherein R^1 is hydrogen.

36. The compound of claim 34 wherein R' is aralkyl.

37. The compound of claim 34 wherein R^1 is heterocyclo lower alkyl or heteroaryl lower alkyl.

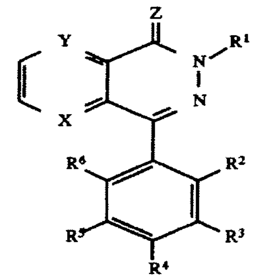
38. The compound of claim 34 wherein R^1 is lower alkyl.

39. The compound of claim 34 wherein R^2, R^4, R^5 and R^6 are hydrogen and R^3 is chloro.

40. The compound of claim 34 wherein R^2, R^4, R^5 and R^6 are hydrogen and R^3 is nitro.

41. The compound of claim 34 wherein R^2, R^3, R^4, R^5 and R^6 are hydrogen.

42. A compound of the formula:



wherein:

X is nitrogen;

Y is carbon;

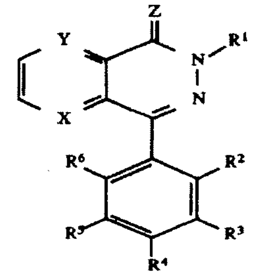
Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo lower alkyl where the heterocyclo is selected from pyrrolidinyl, piperidinyl, N-methylpiperidinyl, or morpholinyl, or heteroaryl lower alkyl (where the heteroaryl is selected from 2-pyridyl, 3-pyridyl, 4-pyridyl, the N-oxides thereof, 2-thienyl, or 3-thienyl, each optionally substituted with halo or lower alkyl);

two adjacent members of R^2, R^3, R^4, R^5 and R^6 taken together are methylenedioxy, and the remaining members are hydrogen;

or a pharmaceutically acceptable salt thereof.

43. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of a compound of the formula:



wherein:

X is nitrogen and Y is carbon, or X is carbon and Y is nitrogen;

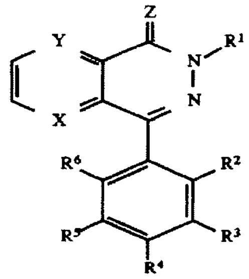
Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo lower alkyl (where the heterocyclo is selected from pyrrolidinyl, piperidinyl, N-methylpiperidinyl, or morpholinyl, or heteroaryl lower alkyl (where the heteroaryl is selected from 2-pyridyl, 3-pyridyl, 4-pyridyl, the N-oxides thereof, 2-thienyl, or 3-thienyl, each optionally substituted with halo or lower alkyl);

R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carbonyl, halocarbonyl, carbamoyl, lower alkyl carbonyl, halocarbonyl, thiomethyl, triflaoromethyl, cyano or nitro;

or a pharmaceutically acceptable salt thereof.

44. A method of treating a disease state selcted from asthma and inflammation in a meal, comprising administering to a mammal in need thereof a therapeutically effective amount of a compound of the formula:



wherein:

X is nitrogen and Y is carbon, or X is carbon and Y is nitrogen;

Z is oxygen or sulfur;

R^1 is hydrogen, lower alkyl, cycloalkyl, cycloalkyl lower alkyl, aryl, aralkyl, heterocyclo lower atkyl (where the heterocyclo is selected from pyrrolidinyl, piperidinyl, N-methylpiperidinyl, or morpholinyl), or heteroaryl lower alkyl (where the heteroaryl is selected from 2-pyridyl, 3-pyridyl, 4-pyridyl, the N-oxides thereof, 2-thienyl, or 3-thienyl, each optionally substituted with halo or lower alkyl);

R^2, R^3, R^4, R^5 and R^6 are independently selected from hydrogen, lower alkyl, halo, carboxy, alkoxycarbonyl, carbamoyl, lower alkyl carbonyl, halocarbonyl, thiomethyl, trifluoromethyl, cyano or nitro;

or a pharmaceutically acceptable salt thereof.

45. The method of claim **44** where the disease state is asthma.

* * * * *